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EFFECTS OF TEMPERATURE ON PRODUCTIVITY AND GENETIC VARIANCE OF BODY SIZE IN POPULATIONS OF DROSOPHILA PSEUDOOBSCURA[1]

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HERITABILITY of body size, i.e., wing and thorax length, has been thoroughly studied by ROBERTSON and REEVE (1952) and TANTAWY (1959) working on *Drosophila melanogaster*. They reported that the heritability of wing length ranged between 30–40 percent. No estimates for body size on other species of Drosophila have been made.

The most important factors that may affect the additive genetic variance of a given character are selection and inbreeding. The effects of selection depend greatly on the parental relationship. Mating between remote relatives causes an increase (REEVE and ROBERTSON 1953; TANTAWY 1956), while that between full sibs causes a decline (TANTAWY 1959). Inbreeding with random mating is the major factor decreasing the additive genetic variance, and the extent to which this may happen depends on closeness of relationship of individuals. TANTAWY and REEVE (1956) and TANTAWY (1957a) reported that matings between full sibs are most effective in depleting the original heterozygosity for a given character, while crossing highly inbred lines (TANTAWY 1957b) leads to increase.

The present investigation was carried out to study the heritability estimates for body size, i.e., wing and thorax length, in populations of *Drosophila pseudoobscura* and the influence of the original temperature conditions on such estimates.

TECHNICAL PROCEDURE

Two cages, Nos. 3 and 6 of populations of *Drosophila pseudoobscura* homozygous for the Arrowhead (*AR/AR*) gene arrangement in the third chromosome, were the material of the present study. The cages were maintained for two years at 25°C and 15°C, respectively. From each cage, 200 virgin females were measured for wing and thorax length, and selection was carried out for large, medium and small wing length. Thirty females from each selected group were used for laying eggs for their lifetime at 25°C and at 15°C. For more details concerning measuring of adult females, methods of selection and egg counting, see TANTAWY and VETUKHIV (1960).

Eggs from the first ten largest females and the first ten smallest females of the

[1] The present work has been carried out during the tenure of the author of the Boese Fellowship at the Department of Zoology, Columbia University, N.Y., U.S.A.

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large and small selected groups were cultured in food bottles. Wing and thorax length of the offspring of both sexes that hatched on the first two days were measured. On each day five males and five females were measured, i.e., 200 pairs of flies were used in each of the selected groups. At the same time a control stock kept in mass matings from each cage was maintained under each temperature and given the same treatment as those of the selected parents. Twenty pairs of flies, on the same two days, were measured from each of the control stocks. Methods recommended by ROBERTSON and REEVE (1952) for measuring wing length and control of environmental variations were followed.

Effect of size on the productivity under various temperatures: The correlation coefficients between parental female size, at various degrees of temperature, and lifetime egg production and longevity are presented in Table 1.

TABLE 1

Phenotypic correlation coefficients between wing length (W) *of the parental females, thorax length* (T), *lifetime egg production* (G) *and longevity* (L), *with their standard errors. Degrees of freedom=89*

Correlation coefficients between	25°C		15°C	
	Cage no. 3	Cage no. 6	Cage no. 3	Cage no. 6
Female wing length and:				
Female thorax length (r_{WT})	0.850 ± 0.029	0.865 ± 0.027	0.941 ± 0.012	0.899 ± 0.020
Egg production (r_{WG})	0.506 ± 0.079	0.263 ± 0.099	0.540 ± 0.075	0.323 ± 0.095
Longevity (r_{WL})	0.143 ± 0.104	0.233 ± 0.100	0.340 ± 0.094	0.267 ± 0.099
Female thorax length and:				
Egg production (r_{TG})	0.621 ± 0.065	0.313 ± 0.096	0.580 ± 0.070	0.369 ± 0.092
Longevity (r_{TL})	0.283 ± 0.088	0.296 ± 0.097	0.436 ± 0.086	0.326 ± 0.095
Egg production and:				
Longevity (r_{GL})	0.728 ± 0.049	0.558 ± 0.073	0.737 ± 0.048	0.763 ± 0.044

The path coefficient theory of WRIGHT (1921a, b, 1923, 1934) was applied to the correlations in Table 1. The points that need to be taken into consideration are:

A. Egg production may be influenced by longevity.

B. Wing and thorax length are influenced by genetic and environmental factors.

C. The genetic factors for body growth may well influence the rate of lifetime egg production positively. Common genetic factors for body growth and longevity seem less probable though not, of course, impossible.

D. Aspects of favorable environmental conditions on body growth may be expected to be favorable also for longevity and for rate of egg production.

One and two factor hypotheses: From Table 1 it can be seen that there are significant differences among the sets in all the correlations except r_{WL} and r_{TL}. There seems to be some tendency toward higher correlations at 15°C than at 25°C, and perhaps some tendency for Cage 3 to show higher correlations than Cage 6, but neither difference is constant. There is enough similarity among the

four sets to make it seem worthwhile to get a single over-all picture from the average correlations. The statistical analyses for all four populations and the set of average correlations are shown in Table 2.

The analysis on the basis of a single common factor requires that thorax length be an almost perfect indicator of the general size. Factor S shows a strong effect on wing at all temperatures, but shows rather varied influence on lifetime egg production. Results are shown in the calculated path coefficients of Table 2. Effects of S on egg production through its effect on longevity are: 0.140 and 0.144 for females of Cages 3 and 6, respectively, which were maintained at 25°C. The corresponding figures for the cages kept at 15°C are: 0.240 and 0.223, respectively. Such results indicate clearly that factor S affects life egg production by this route more at a colder temperature than at a warmer one.

On calculating the correlations from the estimated path coefficients r_{WT}, r_{TG} and r_{GL} agree exactly while, as noted, small deviations from the observed values are shown in the other three. Only in the case of the first population (A) is the deviation as much as half the standard error. This solution must thus be considered as a possible one. The fact that it fits reasonably well does not, however, prove that it is correct. It would, indeed, be very surprising if wing (W), thorax (T) and egg production (G) were acted upon by all factors (genetic and environmental) in such closely parallel fashion that these factors could be combined accurately into one (S_1). Diagram 1, which is based on the average of all four populations, illustrates the effects of such a factor. S_1 affects all four different characters independently and also affects (G) through (L). Such a diagram indicates that the contribution to the correlation of (W) with (G) through (S_1) alone is 0.252 and that through S_1 and (L) is 0.166, which give a total correlation coefficient (r_{WG}) of 0.418. The corresponding figures for the contributions to the correlations of (T) with (G) are: 0.285 and 0.186, respectively, totalling 0.471, which indicate that (W) and (T) show rather similar relations to the egg production rate.

A two factor solution introduces too many paths for a unique solution. It is of interest, however, to find the range of possible solutions. Consideration of equation (11) in Table 2, shows that exact solution may be obtained either by introducing an additional factor common to (W) and (T) or one common to (T) and (G), while an additional one common to (W) and (G) would make the fit worse. The same conclusion follows on making the calculations from equations (1), (3) and (5) instead of equations (1), (2) and (4). It seems better to postulate a factor (S_2) common to wing and thorax, than one (S_3) common to thorax and egg production. However, the latter was also tried out. A unique solution can be obtained (by iteration) by assuming that egg production is related to the additional factor (S_3) only through longevity $(P_{gs_3} = 0)$ as shown in Diagram 2. Another unique solution can be obtained by making P_{gs_3} as large as possible without making S_{WX} impossible $(P_{WX} = 0)$ as shown in Diagram 3. The solutions for the average of correlations under both of these assumptions are reported in Table 3. Such results seem to be of interest only as mathematically possible solutions, rather than as physiologically probable ones.

TABLE 2

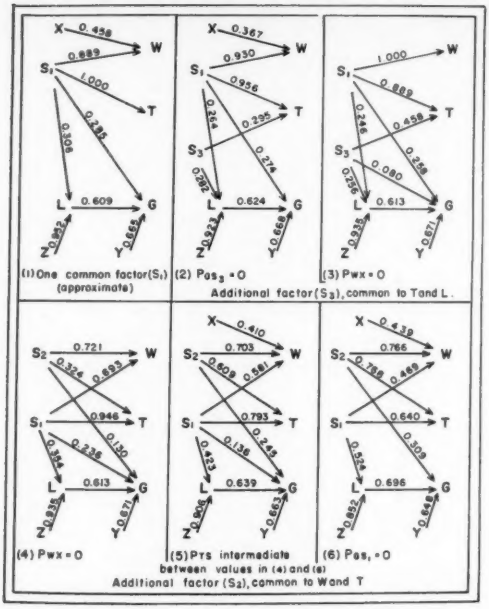
Statistical analyses, for correlations of Table 1, for all four different populations and the set of average correlations

Item	25°C A Cage no. 3	25°C B Cage no. 6	15°C C Cage no. 3	15°C D Cage no. 6	Average correlations
(1) $r_{WT} = P_{WS} P_{TS}$	0.850	0.865	0.941	0.899	0.889
(2) $r_{WG} = P_{WS} r_{GS}$	0.506(0.528)	0.263(0.271)	0.540(0.546)	0.323(0.332)	0.408(0.418)
(3) $r_{WL} = P_{WS} P_{LS}$	0.143(0.192)	0.233(0.244)	0.340(0.375)	0.267(0.279)	0.246(0.272)
(4) $r_{TG} = P_{TS} r_{GS}$	0.621	0.313	0.580	0.369	0.471
(5) $r_{TL} = P_{TS} P_{LS}$	0.283(0.226)	0.296(0.283)	0.436(0.399)	0.326(0.311)	0.335(0.306)
(6) $r_{GL} = P_{GL} + P_{GS} P_{LS}$	0.728	0.558	0.737	0.763	0.696
(7) $P_{WS}/P_{TS} = (2)/(4)$	0.815	0.839	0.931	0.874	0.866
(8) $P_{WS}/P_{TS} = (3)/(5)$	0.503	0.786	0.780	0.819	0.732
(7) and (8) are inconsistent (especially in A, less in C, only slightly in B and D)					
(7) based on the larger correlations will be used to obtain an approximate solution					
(9) $P^2_{WS} = (1) \times (7)$	0.693	0.726	0.876	0.787	0.770
(10) $P_{WS} = \sqrt{(9)}$	0.832	0.852	0.936	0.887	0.878
(11) $P_{TS} = (1)/(10)$	1.022	1.015	1.005	1.015	1.013

It is impossible for P_{TS} to exceed 1.0 under the postulated system of relations. It is, however, remarkable that all populations give values so close to 1.0. A good approximation should be obtained from $P_{TS} = 1.0$.

(12) P_{WS} (revised) = (1) if $P_{TS} = 1.000$	0.850	0.865	0.941	0.899	0.889
(13) $P_{LS} = (3)/(12)$	0.168	0.269	0.362	0.296	0.276
(14) $P_{LS} = (5)$	0.283	0.296	0.436	0.326	0.335
(13) and (14) reflect the inconsistency of (8) with (7). The average will be used.					
(15) P_{LS} (average) = ½ [(13) + (14)]	0.226	0.283	0.399	0.311	0.306
(16) $r_{GS} = r_{TG} = P_{GS} + P_{LS} P_{GL}$	0.621	0.313	0.580	0.369	0.471
(17) $P_{GL}(1 - P^2_{LS}) = (6) - (15)(16)$	0.588	0.469	0.505	0.648	0.552
(18) $P_{GL} = (17)/[1 - (15)^2]$	0.619	0.509	0.601	0.718	0.609
(19) $P_{GS} = (16) - (15)(18)$	0.482	0.169	0.340	0.146	0.289
Calculated path coefficients					
$P_{WS} = (12)$	0.850	0.865	0.941	0.899	0.889
$P_{WX} = \sqrt{1 - (12)^2}$	0.526	0.502	0.339	0.436	0.458
P_{TS} see (11)	1.000	1.000	1.000	1.000	1.000
$P_{GS} = (19)$	0.482	0.169	0.340	0.146	0.285
$P_{GL} = (18)$	0.619	0.509	0.601	0.718	0.609
$P_{GY} = \sqrt{1 - (19)(16) - (18)(6)}$	0.500	0.814	0.600	0.631	0.665
$P_{LS} = (15)$	0.226	0.283	0.399	0.311	0.306
$P_{LZ} = \sqrt{1 - (15)^2}$	0.974	0.959	0.917	0.950	0.952

On substituting in (1) to (6), using $r_{GS} = r_{TG} = (4)$; (1), (4) and (6) agree perfectly with the observed correlations while (2), (3) and (5) come out as indicated in parentheses. The deviations are all small compared with the standard error (which are largest in (3), next in (5) and next to this in (2)).


(1) One common factor (S_1) (approximate)
(2) $P_{os_3} = 0$
(3) $P_{wx} = 0$
Additional factor (S_3), common to T and L.
(4) $P_{wx} = 0$
(5) P_{TS} intermediate between values in (4) and (6)
(6) $P_{os_1} = 0$
Additional factor (S_2), common to W and T

DIAGRAMS 1–6.—Illustrates the different hypotheses derived by the path coefficient theory, where S_1, S_2 and S_3 are size factors, W = wing length, T = thorax length, G = egg production, L = longevity and X, Z and Y are the residuals. Note: (5) P_{TS} should read (5) P_{TS_1}.

The calculated coefficients with additional factor (S_2) common to wing and thorax are given in Table 4a while 4b contains the direct and indirect paths connecting T and G and their total. Diagrams 2 to 6 compare various solutions based on the average correlations. While solutions are possible for all values of P_{WX} between 0.367 (Diagram 2) and 0 (Diagram 3), these do not differ very much in other respects. As noted, these solutions do not seem plausible. Solutions are also possible for all values of P_{WX} between 0 (Diagram 4) and 0.439 (Diagram 6) of which Diagram 5 is one example (exactly intermediate in P_{TS_1}). Diagram 6 with P_{os_2} maximum and $P_{os_1} = 0$ is not very plausible. The most plausible solution is probably somewhere between Diagrams 4 and 5. The former does not differ very much from Diagram 1, except in postulating that the residual factor for *(W)*, (here S_2), has small effects on *(T)* and *(G)* instead of being restricted to action on *(W)*, (factor x).

Heritability of wing and thorax length: It is of interest to relate heritability estimates to the set of path coefficients. This can be done if we take Diagram 4 or 5 as the most plausible and use the same diagram for both parent and offspring (which may not be wholly justified) and if then we determine what the parent-offspring correlation would be on the assumption of no dominance or interaction,

TABLE 3

Illustrates the various correlation coefficients between different characters as well as path coefficients on the assumptions that $P_{GS_3} = 0$ *and* $P_{WX} = 0$. *The approximate one factor (No* S_3*) is given for comparison*

Correlations	Average correlations
$r_{WT} = P_{WS_1} P_{TS_1}$	0.889
$r_{WG} = P_{WS_1} r_{GS_1}$	0.408
$r_{WL} = P_{WS_1} P_{LS_1}$	0.246
$r_{TG} = P_{TS_1} r_{GS_1} + P_{TS_3} r_{GS_3}$	0.471
$r_{TL} = P_{TS_1} P_{LS_1} + P_{TS_3} P_{LS_3}$	0.335
$r_{GL} = P_{GL} + P_{GS_1} P_{LS_1} + P_{GS_3} P_{LS_3}$	0.696
$r_{GS_1} = P_{GS_1} + P_{GL} P_{LS_1}$	
$r_{GS_3} = P_{GS_3} + P_{GL} P_{LS_3}$	
$r_{WW} = P^2_{WS_1} + P^2_{WX}$	1.000
$r_{TT} = P^2_{TS_1} + P^2_{TS_3}$	1.000
$r_{GG} = P_{GS_1} r_{GS_1} + P_{GS_3} r_{GS_3} + P_{GL} r_{GL} + P^2_{GY}$	1.000
$r_{LL} = P^2_{LS_1} + P^2_{LS_3} + P^2_{LE}$	1.000

	No S_3 One factor (approximate)	$P_{GS_3} = 0$	$P_{WX} = 0$
P_{WS_1}	0.889	0.930	1.000
P_{WX}	0.458	0.367	0
P_{TS_1}	1.000	0.956	0.889
P_{TS_3}	0	0.295	0.458
P_{GS_1}	0.285	0.274	0.258
P_{GS_3}	0	0	0.079
P_{GL}	0.609	0.624	0.613
P_{GY}	0.665	0.668	0.671
P_{LS_1}	0.306	0.264	0.246
P_{LS_3}	0	0.282	0.256
P_{LE}	0.952	0.923	0.935
r_{GS_1}	0.471	0.439	0.408
r_{GS_3}	0	0.176	0.236

Two solutions on the assumption of a factor, (S_3), common to T and L in addition to one, (S_1), common to W, T and L. One solution is obtained by assuming that $P_{GS_3} = 0$, the other by assuming that $P_{WX} = 0$. Intermediate solutions can be obtained, without giving any negative values, by taking values of P_{GS_3} between zero and 0.0799. The approximate one factor solution (No S_3) is given for comparison (see Table 2).

plus the further assumptions that S_2 is wholly genetic and S_1 wholly environmental in Diagram 4, or S_2 and S_X are wholly genetic and S_1 environmental in Diagram 5. The results are presented in Diagram 7a and b. Diagram 7a gives a fairly good result for $r_{w_o w_p}$ but too small a result for $T_o T_p$, while Diagram 7b gives considerably too large a result in both cases. A system differing slightly from (a) in the direction of (b) would give the closest fit. There are, however, a good many assumptions made, and it may be doubted whether one is justified in doing more than give the heritabilities as twice the observed parent-offspring correlations on the assumption of no dominance and interaction. Table 5 shows

TABLE 4

(a) The calculated coefficients with an additional factor (S_s) common to wing and thorax for the four different populations and their averages

Item	25°C A Cage no. 3	25°C B Cage no. 6	15°C C Cage no. 3	15°C D Cage no. 6	Average correlations
$r_{WT} = P_{WS_1} P_{TS_1} + P_{WS_2} P_{TS_2}$	0.850	0.865	0.941	0.899	0.889
$r_{WG} = P_{WS_1} r_{GS_1} + P_{WS_2} P_{GS_2}$	0.506	0.263	0.540	0.323	0.408
$r_{WL} = P_{WS_1} P_{LS_1}$	0.143	0.233	0.340	0.267	0.246
$r_{TG} = P_{TS_1} r_{GS_1} + P_{TS_2} P_{GS_2}$	0.621	0.313	0.580	0.369	0.471
$r_{TL} = P_{TS_1} P_{LS_1}$	0.283	0.296	0.436	0.326	0.335
$r_{GL} = P_{GS_1} P_{LS_1} + P_{GL}$	0.728	0.558	0.737	0.763	0.696
$r_{GS_1} = P_{GS_1} + P_{GL} P_{LS_1}$					
$r_{WW} = P^2_{WS_1} + P^2_{WS_2} + P^2_{WX}$	1.000	1.000	1.000	1.000	1.000
$r_{TT} = P^2_{TS_1} + P^2_{TS_2}$	1.000	1.000	1.000	1.000	1.000
$r_{GG} = P_{GS_1} r_{GS_1} + P^2_{GS_2} + P_{GL} r_{GL} + P^2_{GY}$	1.000	1.000	1.000	1.000	1.000
$r_{LL} = P^2_{LS_1} + P^2_{LZ}$	1.000	1.000	1.000	1.000	1.000

	$P_{GS_1}=0$	Int.	$P_{WX}=0$	$P_{GS_1}=0$	Int.	$P_{WX}=0$	$P_{GS_1}=0$	Int.	$P_{WX}=0$	$P_{GS_1}=0$	Int.	$P_{WX}=0$	$P_{GS_1}=0$	Int.	$P_{WX}=0$
P_{WS_1}	0.254	0.337	0.419	0.432	0.604	0.776	0.566	0.635	0.705	0.590	0.698	0.805	0.469	0.581	0.693
P_{WS_2}	0.837	0.841	0.908	0.751	0.626	0.630	0.771	0.729	0.709	0.684	0.583	0.593	0.766	0.703	0.721
P_{WX}	0.485	0.423	0	0.499	0.493	0+	0.294	0.254	0+	0.428	0.416	0	0.439	0.410	0
P_{TS_1}	0.506	0.670	0.835	0.550	0.769	0.988	0.725	0.814	0.904	0.721	0.832	0.983	0.640	0.793	0.946
P_{TS_2}	0.863	0.742	0.551	0.835	0.639	0.156	0.689	0.581	0.428	0.693	0.524	0.183	0.768	0.609	0.324
P_{GS_1}	0	0.196	0.333	0	0.094	0.159	0+	0.129	0.229	0+	0.071	0.129	0	0.136	0.236
P_{GS_2}	0.481	0.414	0.307	0.178	0.136	0.033	0.376	0.317	0.234	0.175	0.132	0.046	0.309	0.245	0.130
P_{GL}	0.728	0.645	0.615	0.558	0.522	0.509	0.737	0.668	0.626	0.763	0.736	0.721	0.696	0.639	0.614
r_{GY}	0.489	0.517	0.527	0.811	0.814	0.815	0.562	0.588	0.602	0.622	0.629	0.633	0.648	0.663	0.671
P_{LS_1}	0.561	0.423	0.339	0.538	0.385	0.299	0.602	0.536	0.483	0.452	0.382	0.331	0.524	0.423	0.354
P_{LZ}	0.828	0.906	0.941	0.843	0.923	0.954	0.799	0.844	0.876	0.892	0.924	0.944	0.852	0.906	0.935
r_{GS_1}	0.408	0.468	0.542	0.300	0.295	0.312	0.443	0.487	0.531	0.345	0.352	0.367	0.365	0.406	0.453

(b) Direct and indirect paths connecting T *and* G *and their total (assuming factor* S_s*)*

$P_T(S_1)G = P_{TS_1} P_{GS_1}$	0	0.131	0.278	0	0.072	0.157	0	0.105	0.207	0	0.061	0.126	0	0.108	0.224
$P_T(S_1L)G = P_{TS_1} P_{GL} P_{LS_1}$	0.206	0.183	0.174	0.165	0.154	0.151	0.321	0.291	0.273	0.249	0.239	0.235	0.233	0.214	0.205
Total $= P_{TS_1} r_{GS_1}$	0.206	0.314	0.452	0.165	0.226	0.308	0.321	0.396	0.480	0.249	0.300	0.361	0.233	0.322	0.429
$P_T(S_2)G = P_{TS_2} P_{GS_2}$	0.415	0.307	0.169	0.148	0.087	0.005	0.259	0.184	0.099	0.121	0.069	0.008	0.238	0.149	0.042
r_{TG}	0.621	0.621	0.621	0.313	0.313	0.313	0.579	0.580	0.580	0.369	0.369	0.369	0.471	0.471	0.471

TABLE 5

Phenotypic correlation coefficients between parental body size and offspring size. Heritability of wing and thorax length are also given

Item	25°C		15°C	
	Cage no. 3	Cage no. 6	Cage no. 3	Cage no. 6
Correlations				
W_pW_o	0.240	0.189	0.203	0.206
T_pT_o	0.111	0.121	0.101	0.126
Heritabilities				
h^2_W	0.480	0.378	0.406	0.412
h^2_T	0.222	0.242	0.202	0.252

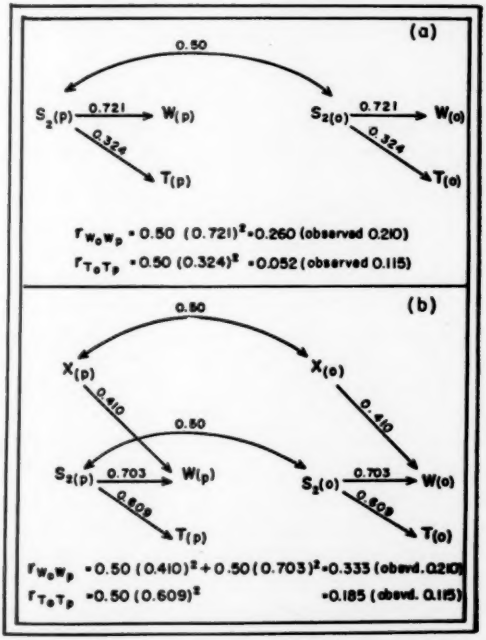


DIAGRAM 7.—Illustrates the relationship between parent and offspring size. For symbols see Diagram 1.

the phenotypic correlation coefficients between parental and offspring size; heritability of wing and thorax length are also reported. The results, as presented in Table 5, indicate that wing and thorax length of *Drosophila pseudoobscura* possess a considerable amount of additive genetic variance under any given temperature with insignificant differences between various degrees.

The results indicate that with changes in the original temperature heritability

estimates decline. The percentage decrease in the heritability estimates for wing length are 15.9 percent and 8.3 percent for Cage nos. 3 and 6, respectively. Such results indicate that the additive genetic variance of a given trait may be affected more by changing temperature from warm to cold conditions, rather than the reverse situation.

Relation between parental female size and offspring size: It has been shown by various investigators that selection of parents for wing length and thorax length has an effect on the progeny (ROBERTSON and REEVE 1952 and later; TANTAWY 1959 on *Drosophila melanogaster;* PREVOSTI 1955 on *Drosophila subobscura*). Selection was effective in all cases, and minus selection shows more response than that in the plus direction.

In the present investigation the progeny of the selected females were measured for wing length and thorax length, and the results are reported in Table 6. Results

TABLE 6

Wing length in the selected offspring and the controls (sexes averaged) at different temperatures with their standard errors. Units of measurements are of 1/100 mm. Response to selection (sexes averaged) as percentage deviations from controls

Cage no.	Temperature	Controls	Offspring		Percent deviations from controls
		Wing (sexes avg.)	Size	Wing (sexes avg.)	
3	25°C.	199.81 ± 1.09	Large	200.64 ± 1.08	+ 0.42
			Small	198.47 ± 1.19	− 0.68
6	25°C.	199.73 ± 1.11	Large	200.17 ± 1.03	+ 0.22
			Small	198.99 ± 1.03	− 0.38
3	15°C.	226.16 ± 1.45	Large	226.80 ± 1.70	+ 0.28
			Small	225.16 ± 1.14	− 0.44
6	15°C.	229.05 ± 1.27	Large	229.86 ± 1.35	+ 0.36
			Small	227.14 ± 2.06	− 0.84

obtained for thorax length are not given since they show a similar behavior to wing length.

The results as presented in Table 6 indicate that the response to selection should be more pronounced if male parents were also selected. The results are presented most effectively as percentage deviations from controls. Response to selection in the minus direction is greater than that in the plus one, thus agreeing with the results of other investigators. The results also show that populations under their original temperature show almost the same response to selection. This may indicate that response to selection, measured as percentage deviations from controls, does not differ significantly from one population to another of the same species, provided they were selected under their original temperature conditions. Such results raise interesting questions which should be investigated, such as the effects of the original temperature conditions on natural populations of Drosophila.

CONCLUSIONS

The path coefficients analyses clearly indicate that body size in *Drosophila pseudoobscura* has definite effects on lifetime egg production. Such effects may differ according to the original temperature under which the populations have been kept for long time. TANTAWY and MALLAH (1961) found, in natural populations of *Drosophila melanogaster* and *D. simulans*, that the original climatic conditions have significant effects in determining various quantitative characters, and TANTAWY and VETUKHIV (1960) reported significant effects of temperature on egg laying capacity in populations of *Drosophila pseudoobscura*.

The present analyses indicate that egg production is a character which is influenced more by environmental conditions than by genetic agencies. ROBERTSON (1957) stated that nonadditive effects predominate in egg production in *Drosophila melanogaster*, while the situation is reversed in the inheritance of body size. Body size in *Drosophila pseudoobscura* behaves in the same manner as in *Drosophila melanogaster* and yields similar heritability estimates. The higher heritability estimates found in the present study indicate that selection in *Drosophila pseudoobscura* should be effective in increasing and decreasing body size.

Changes of the original temperature result in decline of the heritability estimates. This is expected on the basis that selection acts mainly on the additive genetic variance. Since heritability is defined as a ratio of the additive genetic variance to the total variation, it may be altered when either the numerator or the denominator is changed. Therefore, one may conclude that the decline in the heritability estimates in the present study is due to the increase of environmental variations as well as of the environment-genotype interaction, under the new conditions.

SUMMARY

1. An experiment was designed to study the effects of different temperatures and parental female sizes on the egg production rates in *Drosophila pseudoobscura*. Heritability of the body size and the response to selection in one generation were investigated.

2. The results show that temperature and body size affect the rate of egg production and the longevity. The path coefficient theory has been applied to the correlation coefficients between different characters.

3. The correlations between parental size and offspring size indicate that populations of *Drosophila pseudoobscura* possess a considerable amount of additive genetic variance for body size.

4. Changing the original temperature under which the populations have been maintained for a long time results in decline of the heritability estimates; this is followed by a decrease in the response to selection.

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THE GENETIC LOAD IN THE SECOND CHROMOSOMES OF SOME POPULATIONS OF DROSOPHILA MELANOGASTER IN EGYPT[1]

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SINCE the pioneering work of CHETVERIKOV (1927), species of the genus Drosophila, and particularly *Drosophila melanogaster*, have been favorite materials for studies on the genetic loads which populations of sexually reproducing species carry (DOBZHANSKY 1951 and 1955 for reviews). *Drosophila melanogaster* has been used for many studies on the genetics of irradiated and nonirradiated experimental populations (WALLACE 1956 and other works). Nevertheless, much remains to be learned about the behavior of the genetic loads in this species. For one thing, the populations of different countries often differ in that some of them carry more concealed recessive lethal and semilethal genes in their chromosomes than is the case with populations of other countries. This difference probably depends on the environmental conditions in which the populations live, but the nature of the relations are as yet obscure. Moreover, in contrast to some other species (e.g., *D. pseudoobscura*, *D. persimilis*, and *D. prosaltans*, see DOBZHANSKY and SPASSKY 1954), data seem to be wholly lacking on the frequency of recessive subvital variants in wild populations of *D. melanogaster*. The experiments described in the present article were made for the purpose of filling these gaps.

MATERIALS AND METHODS

Samples of the populations of *Drosophila melanogaster* were taken in three localities in Lower Egypt, namely Mehalla El-Kubra (July 27th), Wadi el Natroun (September 9th), and Nubaria (September 26th) all in 1957. In all three places flourishing populations were found, consisting chiefly of *D. melanogaster* (majority) and *D. simulans* (minority).

The technique of analysis recommended by WALLACE (1956) has been used. Wild males, or sons of wild females, were crossed to females of the Curly (*Cy*) Lobe[4] (L^4) Plum (*Pm*) analyzer stock, kindly provided by DR. R. E. SCOSSIROLI of the Istituto di Genetics, Pavia, Italy. A single CyL^4 male was taken from each set of progeny and crossed to CyL^4Pm females. CyL^4 females and *Pm* males were selected in each generation and inbred. In the next generation (F_3) the expected segregation is 25 percent wild type (homozygous for a second chromosome derived from the wild male), 25 percent *Pm*, 25 percent CyL^4, and 25 percent CyL^4Pm. The cultures of the F_3 generation were obtained from ten virgin CyL^4

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females and a like number of *Pm* males. The ten pairs of flies so constituted were aged together for about four days in vials with food, and thereupon transferred three times, at 24-hour intervals, to fresh culture bottles seeded with yeast. This means that the females were permitted to oviposit for 24 hours in each culture, thus producing three replicate cultures. All the cultures were kept in a constant temperature room at 25° ± 1°C. The flies that hatched were counted four times at two-day intervals.

Control experiments: The mutants *Cy*, L^4, and *Pm* used in our experiments may reduce the viability of their carriers below normal. Following DOBZHANSKY, HOLZ, and SPASSKY (1942) we define as normal for our purposes the average viability of individuals which carry the two members of a pair of second chromosomes taken at random from the population of the same geographical locality. To arrive at a standard of the normal viability, groups of ten virgin $CyL^4/+$ females which carried a wild second chromosome derived from a certain wild progenitor were crossed to ten $Pm/+$ males carrying a wild second chromosome from another progenitor. The progenies of these intercrossings were counted and scored exactly like the corresponding progenies of the crosses which produced individuals homozygous for the wild second chromosomes. In all, 95 intercrosses were made from the Mehalla population, 101 from Wadi el Natroun, and 105 from Nubaria. The results are summarized in Table 1.

It can be seen that the percentages of the wild-type class, which in these cultures has by definition the normal viability, is consistently above the theoretical 25 percent in the total counts. Conversely, the frequencies of the CyL^4 and

TABLE 1

The control experiment. Numbers and percentages (%) of four classes of the progeny at different periods of eclosion of the flies in the cultures. The wild-type flies are here carriers of two different second chromosomes taken at random from the population

Locality and count	Wild type		*Pm*		*Cy* L^4		*Cy* L^4/*Pm*	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Mehalla 1st	10549	28.94	10082	27.66	8216	22.54	7599	20.85
Mehalla 2nd	13019	25.12	12978	25.01	12672	24.45	13157	25.39
Mehalla 3rd	1476	23.43	1231	19.54	1597	25.36	1994	31.66
Mehalla 4th	134	22.04	89	14.64	162	26.64	223	36.68
Total	25178	26.04	24380	25.62	22647	23.79	22973	24.14
Wadi 1st	12026	29.70	11211	27.69	9317	23.01	7938	19.60
Wadi 2nd	13879	25.86	13860	25.82	12804	23.85	13134	24.47
Wadi 3rd	1875	23.08	1872	23.04	2009	24.73	2369	29.16
Wadi 4th	275	20.68	263	19.77	357	26.84	435	32.71
Total	28055	27.07	27206	26.25	24487	23.63	23876	23.04
Nubaria 1st	13470	29.91	12148	26.97	10270	22.80	9148	20.31
Nubaria 2nd	13333	24.87	13178	24.58	13043	24.33	14048	26.21
Nubaria 3rd	767	18.78	828	20.27	1136	27.81	1354	33.51
Nubaria 4th	40	12.31	39	12.00	105	32.31	141	43.38
Total	27610	26.76	26193	25.42	24554	23.83	24691	23.96

CyL^4*Pm* classes are consistently below 25 percent. This amounts to saying that the presence in a fly's genotype of *CyL*^{4}, or of *CyL*^{4}*Pm* mutants, slightly reduces its viability. *Pm* is also slightly below the wild-type class in frequency. It may also be seen that the proportions of wild-type and *Pm* flies are consistently higher, and those of *CyL*^{4} and *CyL*^{4}*Pm* flies consistently lower, in the first than in the later counts. Since the development rate of the wild-type class is normal by definition, it follows that the mutants *Cy* and *L*^{4} produce a slight delay in the development of their carriers.

Viability of homozygotes: The numbers of the second chromosomes tested for their effects on the viability of homozygotes were 95 chromosomes from Mehalla, 101 from Wadi el Natroun, and 105 from Nubaria. The numbers of the flies counted and scored in the F_3 generations of the crosses were 87726, 77538, and 99018 for the three localities, respectively. The average numbers of the flies counted per test of a chromosome were 924, 768, and 924, respectively for the same three populations. These numbers varied from one chromosome to another, but only seldom fell below 300 flies per chromosome. The total output of the flies in the three replicate cultures was always used to characterize the viability effects of a chromosome.

The results are reported in Table 2 in terms of the percentages of the normal viability. To express the observations in these terms, the percentage frequency of the wild-type class observed in every culture was divided by the mean frequency of the wild-type class in the corresponding control experiment reported in Table 1. The mean viability was then calculated as the unweighted mean for the three replicate cultures used for testing a given chromosome (see section on Methods).

Inspection of Table 2 discloses that the viability distribution is clearly bimodal. One mode lies just below the normal viability (the 90–100 percent viability class), and the other mode lies in the lethal and extreme semilethal classes (10–0 percent of the normal viability).

The observed percentages of homozygous lethal chromosomes were 19.0 ± 4.0 in Mehalla, 19.8 ± 4.0 in Wadi el Natroun, and 21.9 ± 4.0 in Nubaria. There are obviously no significant differences between the three localities with respect to the frequencies of lethal chromosomes. The apparently lethal chromosomes may be combined with the extreme semilethal chromosomes (those yielding less than

TABLE 2

Viability of homozygotes for second chromosomes, expressed in percentages of the normal viability; the figures indicate numbers of chromosomes that fall in the different viability classes

		Semilethals					Quasi-normals									
Lethal	0	10	20	30	40	50	60	70	80	90	100	110	120	130	Total
Mehalla	18	1	..	2	..	..	1	2	5	16	35	12	3		95
Wadi	20	9	1	1	2	..	1	2	8	17	29	8	2	1	101
Nubaria	23	6	2	1	1	2		3	6	12	30	16	3		105
Total	61	16	3	4	3	2	2	7	19	45	94	36	8	1	301

ten percent of the normal viability) since the distinction between these classes may not be meaningful. We obtain then the percentages of 20.0 ± 4.1 for Mehalla, 28.7 ± 4.6 for Wadi el Natroun, and 27.6 ± 4.4 for Nubaria. The mean frequency is 25.5 ± 2.5 and the variation between the localities is still not significant.

Counting the semilethals alone, we have 3.2 ± 1.8 percent for Mehalla, 12.9 ± 3.4 for Wadi el Natroun, and 11.4 ± 3.2 for Nubaria. The heterogeneity is just significant, at between the two percent and five percent probability levels. The combined figures for lethals and semilethals (this is probably the most meaningful value) are 22.1 ± 4.2 for Mehalla, 32.7 ± 4.7 for Wadi el Natroun, and 33.3 ± 4.6 percent for Nubaria. The mean is 29.56 ± 2.63 percent, and the differences between the localities are not significant. A comparison of the figures for the Egyptian populations with those from other countries will be made in the discussion.

The mean viabilities for the homozygotes for all the chromosomes taken from the three populations are shown in Table 3. It can be seen that if these populations were to become homozygous for their second chromosomes, the mean viability would be just under two thirds of the normal viability (65.94 ± 2.73 percent). The chromosomes which are neither lethal nor semilethal when homozygous are termed quasi-normal. Homozygosis for only quasi-normal chromosomes would reduce the average viability to 92.45 ± 1.50 percent normal.

Estimated frequencies of subvital and supervital chromosomes: Chromosomes which are neither lethal nor semilethal in homozygous condition are designated quasi-normal (WALLACE and MADDEN 1953). Among the chromosomes analyzed in our experiments 70.4 ± 2.6 percent were quasi-normal. However, as pointed out above, the modal viability of the homozygotes for quasi-normal chromosomes is below the mean normal viability of heterozygotes. Some of the quasi-normal chromosomes are evidently subvital in homozygous condition; others may be normal or even supervital.

The statistical technique to estimate the frequencies of the different classes of quasi-normal chromosomes has been proposed by WALLACE and MADDEN (1953) and DOBZHANSKY and SPASSKY (1953, 1954). It consists in calculating the variances of the viabilities observed among the homozygotes for quasi-normal chromosomes, and comparison of these variances with those found among the heterozygotes, i.e., in the control experiment. First, the crude observed variances, σ^2_{res},

TABLE 3

Mean viability, in percentages of the normal viability, of homozygotes for second chromosomes derived from different populations

Locality	All chromosomes	Quasi-normal
Mehalla	72.6 ± 4.58	92.46 ± 3.07
Wadi Natroun	62.30 ± 4.82	90.56 ± 3.54
Nubaria	62.90 ± 4.83	94.35 ± 2.76
Total	65.94 ± 2.73	92.45 ± 1.50

are calculated. The portion of the variance due to sampling errors, σ_s^2, and due to environmental variations, σ_e^2, are estimated from comparisons of the replicate cultures testing the same chromosome and from the numbers of flies counted. The difference between the two values is the genetic, or "real", variance σ_r^2. A square root of the genetic variance gives the standard deviation, σ_r, due to the genotypic variability in our materials. All these estimates are shown in Table 4.

The frequency of subvital chromosomes is now estimated as the part of the normal distribution with a standard deviation, σ_r of the quasi-normal chromosomes, lying to the left of a value obtained by subtracting two standard deviations, $2\sigma_r$ for the heterozygotes, from the normal viability value. Similarly, the supervital chromosomes lie beyond two standard deviations above the normal viability value. The remainder of the quasi-normal chromosomes are, then "normal". For more details concerning these calculations, see the papers referred to above. The estimates obtained from our material are summarized in Table 5. The mean frequency of subvital chromosomes, among the quasi-normals, is about 42 percent, of normal chromosomes about 54 percent, and of supervital chromosomes about four percent.

Examination of Table 4 discloses a further interesting fact. The portion of the variance due to sampling and to environmental fluctuations (σ_{es}^2) is higher for the homozygotes (average 34.6) than for the heterozygotes (average 25.4). Since the sampling errors were about equal in the two classes of crosses, the difference was evidently in the environmental variance (σ_e^2). This agrees with the results of

TABLE 4

Variances of the viabilities observed in heterozygotes and homozygotes for second chromosomes derived from different populations

Locality	σ^2_{res}	σ^2_{es}	σ^2_r	σ_r
		Heterozygotes		
Mehalla	56.375	23.634	32.741	5.72
Wadi Natroun	34.673	28.182	6.491	2.55
Nubaria	57.754	24.375	33.379	5.79
		Homozygotes		
Mehalla	105.450	37.282	68.168	8.26
Wadi Natroun	147.210	37.071	110.139	10.50
Nubaria	113.771	29.443	84.328	9.18

TABLE 5

Estimated percentages of subvital, normal, and supervital chromosomes among the quasi-normal chromosomes derived from different populations

Locality	Subvital	Normal	Supervital
Mehalla	31.9	67.0	1.1
Wadi Natroun	66.6	25.1	8.2
Nubaria	26.1	70.8	3.1
Mean	41.6	54.3	4.1

DOBZHANSKY and WALLACE (1953) and of DOBZHANSKY, PAVLOSKY, SPASSKY and SPASSKY (1955), who found that the homeostatic buffering in the heterozygotes is generally superior to that in homozygotes.

Sterility; Individuals homozygous for different quasi-normal chromosomes were tested for fertility. The tests were made separately for the homozygous females and for the males. The results are summarized in Table 6. About 12 percent of the homozygotes cause female sterility, and about 15 percent male sterility. A much smaller fraction of the chromosomes, about three percent, make both sexes sterile.

DISCUSSION

The magnitude of the genetic load found in the second chromosomes of the populations of *Drosophila melanogaster* in Egypt may now be compared with that in other populations of the same species and in other species. IVES (1945, 1954) studied the incidence of lethal and semilethal second chromosomes in populations of several localities in the United States, particularly in Massachusetts and Florida. The proportions of such chromosomes were 48.8 ± 2.2 percent in 1938–1946, and 35.9 ± 1.4 percent in 1947–1952 in the Massachusetts population; the figures for the Florida populations were 65.3 ± 2.6 percent in 1940–1942, and 51.1 ± 4.4 percent in 1951. The comparable figure for the Egyptian populations was found to be 29.61 ± 2.6 percent. It is significantly lower than the figures for Florida and the early figures for Massachusetts, and probably also lower than that for the more recent samples from Massachusetts.

On the other hand, the frequencies of lethals seem to be very low in Russian populations (DUBININ 1946). DUBININ's early experiments (1932–1934) yielded figures ranging from 7.9 to 21.4 percent for various populations in the Caucasus, while his later studies (1937–1939) gave between 20.6 and 27.8 percent in the Caucasus, Crimea, and South Russia, with one exceptional sample going as high as 38.9 percent. It is not clear from DUBININ's paper whether his data included only complete lethals or lethal as well as semilethal chromosomes, but in either case his figures seem to be rather consistently lower than ours.

In point of geographic proximity, the populations of Israel studied by GOLDSCHMIDT (1951) and GOLDSCHMIDT, WAHRMAN, LEDERMANN-KLEIN and WEISS (1955) are closest to ours. Their figure for lethals and semilethals was 39.1 ± 1.4

TABLE 6

Percentages of quasi-normal chromosomes which cause sterility of females, or of males, or of both sexes when homozygous

Locality	Chromosomes tested	Female sterile	Male sterile	Both sterile
Mehalla	74	12.2 ± 3.8	14.9 ± 4.1	2.7 ± 1.9
Wadi	69	13.0 ± 4.1	18.8 ± 4.7	2.9 ± 2.0
Nubaria	72	11.1 ± 3.7	12.5 ± 3.9	4.2 ± 2.4
Total	215	12.1 ± 2.2	15.3 ± 2.5	3.3 ± 1.2

percent. This agrees well with IVES's findings for Massachusetts but is significantly higher than our 29.6 ± 2.6 for Egypt. It seems to be clear that the incidence of lethal and semilethal chromosomes is not uniform in populations of different countries.

There seem to be no estimates of the frequencies of subvital chromosomes, and of chromosomes causing sterility, comparable with ours for any other natural populations of *D. melanogaster*. WALLACE and MADDEN found 37 percent subvital second chromosomes (among the quasi-normal ones) in their unirradiated control experimental population of *D. melanogaster*, and only slightly more (37 to 45 percent) in the irradiated populations. This frequency agrees quite well with our average figure (41.6 percent, Table 5). DOBZHANSKY and SPASSKY (1954) give, however, much higher figures for the chromosomes of *D. pseudoobscura* and *D. persimilis* (84.4–99.5 percent), and a lower figure only for *D. prosaltans* (33.4 percent for the second chromosome, which is homologous to the second chromosome of *D. melanogaster*). Our figures for the sterility (Table 6) agree, however, reasonably well with those for *D. pseudoobscura*, *D. persimilis*, and *D. prosaltans* but are much lower than those for *D. willistoni* (DOBZHANSKY and SPASSKY 1954, Table 10).

SUMMARY

Samples of natural populations of *Drosophila melanogaster* were collected in three localities in Egypt. Using the technique of WALLACE and MADDEN, the genetic load carried in the second chromosomes of these three populations was examined.

A mean of 29.56 ± 2.63 percent of the second chromosomes were lethal or semilethal when homozygous. Among the remainder, the quasi-normal chromosomes, an estimated 42 percent were subvital, 54 percent normal, and four percent supervital in homozygous condition. Among the quasi-normal chromosomes, 12.1 ± 2.2 percent produce sterile females, 15.3 ± 2.5 sterile males, and 3.3 ± 1.2 percent sterile individuals of both sexes.

The frequencies of lethal and semilethal chromosomes in the Egyptian populations can be compared with those in populations of other countries. This comparison shows that the loads of the populations examined from some localities in the United States, and also in Israel, appear to be heavier than in Egypt. Conversely the genetic loads in Russian populations are, at least taking the data at face value, lighter than in Egypt.

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MODIFICATION OF ULTRAVIOLET-INDUCED MUTATION FREQUENCY IN NEUROSPORA CRASSA[1]

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IT has been shown that conditions favoring protein synthesis immediately following ultraviolet (UV) irradiation cause a considerable increase in the frequency of induced mutants in bacteria. WITKIN (1956) found that UV-induced back mutation frequencies of *Salmonella typhimurium* and *Escherichia coli* auxotrophs depended on the presence of a pool of amino acids in the incubation medium before the first postirradiation cell division. She postulated a process "X," involving protein synthesis, which must take place before the termination of the sensitive period (time after irradiation during which the mutation yield is subject to modification). This process could be (1) a process of repair of genetic damage, (2) production or decay of an unstable mutagenic intermediate, or (3) production or decay of an unstable condition within the genic material.

SCHWARTZ and STRAUSS (1958) reported similar experiments with *E. coli* using amino acid analogues. Their results suggest that synthesis of normal, active protein is necessary for the observed increase in mutation frequency.

DOUDNEY and HAAS have shown, in a number of studies, the importance of both protein and ribonucleic acid (RNA) synthesis in the induction and expression of bacterial mutants (HAAS and DOUDNEY 1957, 1959; DOUDNEY and HAAS 1958, 1959).

The purpose of the experiments reported here was to determine whether conditions favoring protein synthesis had a similar modifying effect on the UV-induced mutation frequency in *Neurospora crassa.*

MATERIALS AND METHODS

Organisms: The investigation was carried out with a leucine-requiring mutant, *leu,* (isolated in this laboratory) of strain C102-3704-1A of *Neurospora crassa.* Strain C102-3704-1A, obtained from DR. SRB (Cornell University) grows colonially on minimal medium at 35°C, while at 25°C it behaves like a typical wild type. The leucine requirement was induced by UV and showed no spontaneous reversion.

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Growth media: The minimal medium used was that of BEADLE and TATUM (1945). This was supplemented with 100 mg of L-leucine per liter for the growth of the leucine-requiring mutant. Complete medium contained 5 gm of yeast extract and 0.1 gm casein hydrolysate per liter of minimal medium. The buffer used was 0.1 M sodium/potassium phosphate buffer, *p*H 6.0.

Back mutation experiments: To obtain conidia of uniform age for use in a back mutation experiment, the method described by STRAUSS (1958) was used. In a typical experiment the washed conidia were suspended in water so that the suspension contained about $2–4 \times 10^7$ conidia per ml. Seven to ten ml portions of this suspension in open Petri dishes were exposed to UV light for the desired length of time. The source of the UV light was a 30 watt G.E. Germicidal Lamp at a distance of 7 cm from the suspension. The energy output of the lamp at this distance was 118 ergs/sec/mm^2. Five ml samples of the irradiated suspension were then transferred to 250 ml Erlenmeyer flasks containing 35 ml of the desired incubation media. The conidia were incubated for three hours as 35°C, resuspended in water, and appropriate dilutions plated on minimal and on leucine-supplemented plates to give about 100–200 colonies per plate. Six plates of each medium were used for each sample. A nontreated sample of conidia was plated as a control in each experiment. The plates were incubated for 4–6 days at 35°C, and the colonies were counted.

The conidia forming colonies on minimal medium were scored as back mutants, while the number of colonies on leucine-supplemented plates was used to calculate the number of survivors. The number of conidia plated was determined by counting the conidia in an aliquot of each sample, using a hemocytometer counting chamber. The proportion of viable conidia in the suspension was calculated from the number of colonies on the leucine-supplemented plates and the number of conidia plated. The percent survival of a given sample was calculated from the proportion of viable conidia in this sample as compared to that in the nontreated control, the latter being taken as 100 percent. The mutation frequency was expressed as mutants per survivors (average number of colonies on minimal plates divided by the average number of colonies on supplemented plates, corrected for the difference in the dilutions plated) to enable comparison among samples with different levels of survival.

Photoreactivation: The conidia were suspended in the postirradiation incubation media during photoreactivation. The source of light was a 100 watt light bulb, placed 14 cm below the flasks. A temperature of 30–35°C was maintained during the photoreactivation to allow simultaneous protein synthesis.

RESULTS

A number of control experiments were carried out to show that the results could not be biased by the methods used. Various dilutions of a suspension of *leu* conidia were added to a suspension of wild-type (C102-3704-1A) conidia and the mixtures plated on minimal medium to determine the extent of "crowding" of wild-type colonies by *leu* conidia (GRIGG 1952, 1958). It was found that the number of colonies remained constant when up to 10^7 *leu* conidia were plated

per plate. Since in the back mutation experiments the total number of conidia per plate was approximately 10^6, the possibility of crowding in these experiments must be ruled out.

Since the postirradiation treatments were carried out by incubating the conidia in various media, it was necessary to show that this incubation did not result in selection for or against the revertant conidia. Revertant and *leu* conidia were mixed in known proportions and the mixtures plated either directly or after incubation for three hours. Although the results showed that incubation in leucine-supplemented medium increased the viability of both kinds of conidia. neither kind had any selective advantage over the other. Similarly, revertant conidia formed colonies on minimal and on supplemented plates with the same efficiency.

Incubation for three hours at 35°C before plating was sufficient to obtain the maximum effect on mutation frequency but resulted in no visible germination of the conidia. Incubation of the plates for 3–4 days allowed the expression of all viable conidia. Further incubation resulted in no increase in the number of colonies.

Effect of protein synthesis on the back mutation frequency after a UV dose of 30 seconds: The effects of incubation in complete medium and in minimal medium after 30 sec of UV irradiation are shown in Table 1. These data show that both incubations increased the mutation frequency as compared to the conidia which were plated immediately after irradiation. This increase was smaller when the incubation was in complete medium, and therefore complete medium decreased

TABLE 1

Effect of leucine on the back mutation frequency induced by a UV dose of 30 seconds

Incubation medium		Percent survival	Fraction of viable conidia	Mutants/10^6 survivors
Before UV	After UV			
	none	91	0.56	115
	minimal	68	0.42	181
	complete	69	0.43	130
	none	56	0.19	202
	minimal	54	0.18	295
	min. + leucine	84	0.28	122
	none	95	0.58	96
	buffer	37	0.23	201
	buffer + leucine	49	0.30	170
minimal	none	90	0.31	162
min. + leucine	none	67	0.23	58
minimal	minimal	41	0.14	283
minimal	min. + leucine	68	0.23	221
min. + leucine	minimal	55	0.19	94
min. + leucine	min. + leucine	79	0.27	76

The conidia were irradiated for 30 sec. and incubated in the medium indicated. All incubations were for three hours at 35°C. Data are from four separate experiments.

the induced mutation frequency as compared to samples incubated in minimal medium. A similar decrease was caused by leucine, either in minimal medium or in buffer.

It is interesting to note that the UV-induced back mutation frequency of *leu* was about ten times higher than that of two other Neurospora mutants under similar conditions (VAHARU, unpublished data). Genetic analysis of the revertants showed that they were probably back mutants and not suppressors.

WITKIN (1956) found that when tryptophan-requiring *E. coli* were incubated in a complete medium before irradiation, the postirradiation dependence on amino acids for mutation was more pronounced. It was decided to determine the effects of preirradiation incubation on the UV-induced back mutation frequency of *leu*. From the data in Table 1 it can be seen that leucine decreased the number of back mutants obtained, regardless of whether it was present in the medium before or after irradiation.

As the *leu* strain could not grow in the absence of an exogenous supply of leucine, it was assumed that the addition of leucine to the medium provided the conditions necessary for protein synthesis. This assumption was supported by experiments with chloramphenicol or amino acid analogues (Table 2). Chloramphenicol, known to inhibit protein synthesis in a variety of biological systems (GALE and FOLKES 1953; LEPAGE 1953; BREITMAN and WEBSTER 1958), was found to prevent the decrease in the mutation frequency caused by leucine. In the presence of the amino acid analogues *p*-fluorophenylalanine, ethionine or tryptazan the effect of leucine was also greatly reduced. Only in the presence of 5-methyltryptophan, the analogue which is not incorporated into proteins (SHARON and LIPMAN 1957) and which has been found to preserve the UV-

TABLE 2

Effect of chloramphenicol and amino acid analogues on the back mutation frequency induced by a UV dose of 30 seconds

Incubation medium	Percent survival	Fraction of viable conidia	Mutants/10^6 survivors
minimal	84	0.49	193
minimal + CLM*	85	0.49	164
minimal + leucine	74	0.45	142
minimal + leucine + CLM	65	0.38	175
min. + fluorophenylalanine	106	0.57	151
min. + fluorophenylalanine + leucine	101	0.54	139
minimal + ethionine	98	0.52	138
minimal + ethionine + leucine	115	0.61	134
min. + methyltryptophan	134	0.72	135
min. + methyltryptophan + leucine	133	0.71	114
minimal + tryptazan	111	0.59	113
minimal + tryptazan + leucine	145	0.78	110

The conidia were irradiated for 30 sec. and incubated in the medium indicated. All incubations were for three hours at 35°C.

* CLM = chloramphenicol, 50 μg/ml. Data are from two separate experiments.

TABLE 3

Effect of leucine on the back mutation frequency induced by a UV dose of 60 seconds

Incubation medium	Percent survival	Fraction of viable conidia	Mutants/10^8 survivors
minimal	18	0.07	231
minimal + leucine (10 mg/l)	16	0.06	414
minimal + leucine (50 mg/l)	24	0.09	441
minimal + leucine (100 mg/l)	24	0.09	551
minimal	3	0.02	446
minimal + leucine	4	0.03	854
minimal + leucine + CLM*	6	0.05	409
complete	7	0.05	502

The conidia were irradiated for 60 sec. and incubated in the medium indicated. All incubations were for three hours at 35°C.
* CLM = chloramphenicol, 50 μg/ml. Data are from two separate experiments.

produced sensitive state or "mutagen" in *E. coli* (SCHWARTZ and STRAUSS 1958), was there any measurable lowering of the mutation frequency when leucine was added.

Effect of protein synthesis on the back mutation frequency after a UV dose of 60 seconds: Following a higher dose of UV (60 sec) the effects of the postirradiation incubation media on the mutation frequencies were quite different (Table 3). The presence of leucine in the medium increased the mutation frequency. Complete medium had the same effect as leucine but to a lesser extent. Chloramphenicol again prevented the effect of leucine, suggesting that the effect was due to protein synthesis.

Dependence of the effect of protein synthesis on the dose of UV: When the effect of leucine on the induced mutation frequency was studied as a function of the dose of UV it became evident that following high doses of UV the addition of leucine to the incubation medium increased the mutation frequency, while after low doses the effect was reversed and the mutation frequency was decreased. The effect was found to vary with the dose in both directions, that is, the higher the dose, the greater the increase in mutation frequency caused by leucine (expressed as percent difference between the mutation frequency in samples incubated in minimal medium and in leucine-supplemented medium), the lower the dose, the greater the decrease (Table 4). Similar results were obtained when complete medium was used.

Length of the sensitive period: Experiments were carried out to determine the length of time after irradiation during which the mutation frequency could be modified by the addition of leucine to the incubation medium. The results (Table 5) show that following 30 sec of UV the conidia lost their sensitivity to leucine within the first 15 minutes, while following 60 sec of UV the sensitive period lasted 45 minutes.

Effect of RNA derivatives on the back mutation frequency: HAAS and DOUDNEY (1957) have stressed the importance of RNA synthesis in the establishment of mutants following UV irradiation in *E. coli*. It was decided to determine the effect

TABLE 4

Dependence of the effects of leucine and complete medium on the dose of UV

UV dose (sec)	Incubation medium	Mutants/10^8 survivors	Percent change in mutants/survivors as compared to minimal
10	minimal	131	...
10	minimal + leucine	85	− 35
30	minimal	249	...
30	minimal + leucine	210	− 16
70	minimal	203	...
70	minimal + leucine	575	+183
10	minimal	32	...
10	complete	18	− 44
30	minimal	140	...
30	complete	170	+ 21
50	minimal	322	...
50	complete	427	+ 31
70	minimal	294	...
70	complete	478	+ 63

All incubations were for three hours at 35°C. Data are from two separate experiments.

TABLE 5

Effect of incubation in minimal medium before the addition of leucine on the UV-induced back mutation frequency

UV dose (sec)	Minutes in minimal	Mutants/10^8 survivors	Percent change in mutants/survivors as compared to zero time
30	0	122	..
30	15	139	+14
30	45	142	+16
30	60	143	+17
30	120	149	+22
30	180	152	+25
60	0	717	..
60	15	717	0
60	30	718	0
60	45	715	0
60	60	369	−48
60	180	324	−55

The conidia were irradiated for the time indicated and then suspended in minimal medium (0 time). Leucine was added (to give a concentration of 100 mg/l) after the time indicated. Total incubation was for three hours at 35°C.

of RNA derivatives in the postirradiation incubation media on the back mutation frequency of *leu*. Following 30 sec of UV irradiation both the purine and pyrimidine bases of RNA (adenine, guanine, uracil and cytosine) and the corresponding ribosides were found to increase the mutation frequency when they were added to leucine-supplemented media (Table 6). However, they had no effect when chloramphenicol was also present in the medium. After high doses of UV the RNA bases had very little effect on the mutation frequency.

TABLE 6

Effect of RNA derivatives on the back mutation frequency induced by a UV dose of 30 seconds

Incubation medium	Percent survival	Fraction of viable conidia	Mutants/10^8 survivors
minimal + leucine	84	0.57	134
min. + leucine + RNA bases	62	0.42	174
min. + leucine + ribosides	62	0.42	176
min. + leucine + CLM*	76	0.52	152
min. + leuc. + RNA bases + CLM	62	0.42	153

The conidia were irradiated for 30 sec. and incubated in the medium indicated. All incubations were for three hours at 35°C. RNA bases = mixture of adenine, guanine hydrochloride, uracil and cytosine (0.01 mg/ml of each). Ribosides = mixture of adenosine, guanosine, uridine and cytidine (0.01 mg/ml of each).
*CLM = chloramphenicol, 50 μg/ml.

TABLE 7

Effect of caffeine on the back mutation frequency induced by a UV dose of 30 seconds

Incubation medium	Percent survival	Fraction of viable conidia	Mutants/10^8 survivors
minimal	87	0.38	157
minimal + caffeine	102	0.45	175
minimal + leucine	104	0.46	126
minimal + leucine + caffeine	155	0.68	154
minimal + caffeine + ribosides	88	0.39	177
min. + leuc. + caffeine + ribosides	109	0.48	136

The conidia were irradiated for 30 sec. and incubated in the medium indicated. All incubations were for three hours at 35°C. Concentration of caffeine 0.05 mg/ml. Ribosides = mixture of adenosine, guanosine, uridine and cytidine (0.01 mg/ml of each).

Effect of caffeine on the back mutation frequency: Caffeine, although mutagenic for *E. coli* (NOVICK 1955), did not induce any back mutants in *leu* at a concentration of 0.05 mg/ml. However, it was found to increase the UV-induced mutation frequency when present in the postirradiation incubation medium after 30 sec of irradiation (Table 7). Addition of the ribosides together with caffeine resulted in no further increase in the mutation frequency.

Effect of photoreactivation: DOUDNEY and HAAS (1959) have found a correlation between "mutation fixation" as determined by susceptibility to chloramphenicol and to photoreactivating light. They have further shown (HAAS and DOUDNEY 1960) that mutation susceptibility to chloramphenicol is correlated with RNA synthesis, and that the loss of photoreversibility is correlated with deoxyribonucleic acid (DNA) synthesis in the culture. Since this suggests that protein synthesis and visible light affect different (but related) steps in the UV-induced mutation process, it was of interest to investigate the effects of simultaneous photoreactivation and protein synthesis on the back mutation frequency of *leu* both after low and high doses of UV. Photoreactivation for one hour immediately following irradiation was found to increase the survival and to decrease the induced mutation frequency (Table 8). However, this incubation in the light also counteracted the effects of leucine on the mutation frequency, both after 10 sec

TABLE 8

Effect of photoreactivation on the back mutation frequency

UV dose (sec)	Incubation medium	Condition	Percent survival	Fraction of viable conidia	Mutants/10^6 survivors
10	minimal	dark	71	0.25	63
10	min. + leucine	dark	107	0.37	47
10	minimal	light	95	0.33	32
10	min. + leucine	light	113	0.40	30
60	minimal	dark	23	0.08	302
60	min. + leucine	dark	42	0.15	474
60	minimal	light	101	0.35	295
60	min. + leucine	light	85	0.30	351

The conidia were irradiated for the time indicated and incubated for three hours. Photoreactivated conidia were exposed to visible light during the first hour of incubation, followed by two hours of incubation in the dark.

and 60 sec of UV. After either dose, the mutation frequency in the samples incubated in leucine-supplemented medium approached that obtained in minimal medium.

DISCUSSION

The results show clearly that conditions which affect protein synthesis immediately following UV irradiation can modify the induced mutation frequency in Neurospora. However, there are several differences between the results obtained in this study and those reported for work with *E. coli* (WITKIN 1956; DOUDNEY and HAAS 1958; SCHWARTZ and STRAUSS 1958). While the mutation frequencies could be modified by various postirradiation treatments in both organisms, the magnitude of these effects was much smaller in Neurospora than in *E. coli*. One explanation for this might be that Neurospora has a considerable free amino acid pool whereas this is not true for *E. coli*. The former may be able to synthesize at least some protein even in the absence of exogenous supplies of the required amino acid. This is consistent with the finding that preirradiation incubation of Neurospora conidia in a medium containing leucine had (qualitatively) the same effect as did addition of leucine after irradiation.

The important difference, however, lies in the fact that in these studies protein synthesis after low doses of UV decreased the mutation frequencies, while only increases have been observed in *E. coli*. It is possible that a similar decrease in the mutation frequencies could be found in *E. coli* if the effect of protein synthesis were studied as a function of UV dose and sufficiently low doses were used. On the other hand, this may indicate a real difference between mutation in the two organisms.

HAAS and DOUDNEY (1957), using *E. coli*, showed that the presence of ribosides in the preirradiation incubation medium resulted in increased mutation frequencies when the post irradiation conditions allowed protein synthesis. However, they failed to obtain a similar increase when the ribosides were added after irradiation. In the studies reported here the presence of either RNA bases or ribo-

sides in the postirradiation incubation medium after low doses of UV was found to increase the mutation frequency, provided that some protein synthesis could take place. It might be that this effect, like the effect of protein synthesis, depends on the dose of UV. It is, of course, also possible that the difference may be caused by differences between the two organisms used. The increased UV-induced mutation frequency in the presence of caffeine could be explained by the lengthened sensitive period caused by caffeine (WITKIN 1958).

In these studies no attempt was made to determine the mechanism by which protein synthesis affected the mutation frequency. Similarly, it is not known which steps of the mutation process were affected by the treatments employed. It has been suggested that conditions favoring protein synthesis after UV irradiation may be necessary to prevent the death rather than enhance the establishment of mutations in the genetic material (WITKIN 1956) and that protein synthesis is necessary for mutation expression (HAAS and DOUDNEY 1959).

On the other hand, it can also be assumed that protein synthesis affects the establishment of mutations. DOUDNEY and HAAS (1958) have postulated that some intracellular "mutagen", produced by UV, has to be temporarily stabilized and subsequently fixed by RNA synthesis and that both of these processes depend on a source of amino acids. However, they have also shown that neither of these processes is correlated with the gross protein synthesis in the culture but that there is a close correlation between mutation fixation (as determined by susceptibility to chloramphenicol) and RNA synthesis (DOUDNEY and HAAS 1959).

One possible explanation of the dose-dependence of the effects of protein synthesis might be in terms of the general effects of different doses of UV on the biochemical processes of cells. It is known that the lag in DNA synthesis is roughly proportional to the radiation dose (KANAZIR and ERRERA 1956), and it has been suggested that protein synthesis is necessary to overcome the inhibition (HAROLD and ZIPORIN 1958; DOUDNEY 1959). Since metabolic processes play an important role in the UV-induced mutation process, it might be expected that the effect such processes as nucleic acid and protein synthesis have on mutation would depend on the degree of their inhibition by UV.

SUMMARY

The Neurospora back mutation test was used to study the modifying effects of protein synthesis, immediately following UV irradiation, on the induced back mutation frequency of a leucine-requiring mutant of *Neurospora crassa*. It was found that the effect of protein synthesis depended on the radiation dose. After low doses of UV conditions which favored protein synthesis decreased the mutation frequency, while after high doses the same conditions increased the mutation frequency. These effects were roughly proportional to the dose of UV, and were decreased by simultaneous photoreactivation. Addition of RNA derivatives to the postirradiation incubation medium increased the mutation frequency when the dose of UV was low but had no effect when the dose was high.

It is suggested that the differences between these modifying effects after low

and high doses of UV may be caused by differences in the general effects of different doses of UV on the biochemical processes of the cells.

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CHROMATID ABERRATIONS INDUCED BY GAMMA IRRADIATION. I. THE STRUCTURE AND FREQUENCY OF CHROMATID INTERCHANGES IN DIPLOID AND TETRAPLOID CELLS OF VICIA FABA

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IN recent years a considerable amount of effort has been devoted to the study of the aberrations of chromosome structure induced by ionizing radiations, particularly with reference to quantitative differences due to dose, dose-rate and other controllable variables (see reviews by CATCHESIDE 1948; GILES 1954; and LEA 1955). In such experiments, aberrations involving an exchange of chromatids or parts of chromatids are conveniently classified into two groups, the intrachanges, i.e., those involving exchange within, or between, chromatids within a chromosome, and interchanges, i.e., where the exchange occurs between chromatids of different chromosomes (CATCHESIDE, LEA and THODAY 1946). In *Vicia faba* root-tip cells the chromatid intrachanges induced by ionizing radiation are of at least four different types, and studies on the relative frequencies of these types has led to a new interpretation of the mode of formation of certain classes of chromatid aberrations by ionizing radiation (REVELL 1959). In the present work a study was made of the relative frequencies of the various types of interchanges in diploid roots and in diploid and tetraploid cells of roots subjected to colchicine.

EXPERIMENTAL METHODS

Seeds of *Vicia faba* (Suttons' Prolific Longpod) were germinated and the seedlings cultured in aerated, running tap water at 19°C as described previously (EVANS, NEARY and TONKINSON 1957). On the tenth day after germination the primary roots of the seedlings were irradiated with Co^{60} gamma rays.

For the two hours prior to fixation the roots were placed in nonaerated saturated aqueous solutions of *p*-dichlorobenzene (EVANS and TONKINSON 1959) and fixations of the terminal 2 cms of primary root were made, under vacuum, at intervals between 3½ and 8 hours after irradiation. Fixations were made in a solution made up of two percent osmic acid, two percent chromic acid and distilled water in the ratio (v/v) of 1:1.7:2; after 24 hours the material was thoroughly washed and allowed to bleach and macerate in a 1:6 (v/v) mixture of 40 vol. hydrogen peroxide and ammonium oxalate (saturated aqueous solution) for 20 minutes (C. E. FORD, personal communication). The roots were stained by the Feulgen procedure and the terminal 1.5 mm of each primary root was used to make one squash preparation.

The data on interchange frequency in diploid roots presented in the first part

of this paper is from four separate experiments in which radiation doses of from 51 to 147 rads were used—all the doses being given over a duration of 4½ to 5½ minutes. For the experiment with colchicined roots on the ninth day after germination the seedling roots were treated with an aerated 0.05 percent aqueous solution of colchicine for 3½ hours at 19°C (EVANS and SAVAGE 1959) then thoroughly washed and replaced in tap water at the same temperature. Twenty-five hours after the commencement of the colchicine treatment the roots were irradiated with 118 rads of Co^{60} gamma rays given in 3.8 minutes. The roots were pretreated with *p*-dichlorobenzene and fixed at four, five and six hours after irradiation, as described above. Four slides were scored at each fixation time, and on each slide 200 diploid and 150 tetraploid cells were scored for chromatid interchanges.

All the slides were coded and randomized for each experiment. Less than two percent of the total interchanges observed were rejected as being difficult to score, usually because of the complex nature of the interchange, so that observational bias from this source was negligible.

Experiments with diploid roots

Chromatid interchanges are usually classified on the basis of two characteristics, symmetry and completeness. If the exchange results in the production of a dicentric chromatid and an acentric fragment, or if it is of a type which if completely fulfilled would give rise to a dicentric chromatid, then it is termed asymmetrical (A), as opposed to the symmetrical situation (S) where each chromatid has one centromere. If the exchange is not completely fulfilled, so that of the four broken ends which are assumed to be produced only two undergo union, then the exchange is termed incomplete (I) as opposed to the complete (C) condition where all ends undergo union.

In some of the early experiments carried out in this laboratory it was found that the asymmetrical interchanges occurred more frequently than the symmetrical types. Such an excess of A type over S type interchanges has been noted by a number of authors (Table 1), although LEA (1955), summarizing the data available up until 1946, stated that although in some experiments there was a significant excess of A over S types "it is doubtful if the reliability of the distinction between symmetrical and asymmetrical interchanges is sufficient to make such a difference certain" and suggested that S and A type interchanges were approximately equally frequent.

The separation of interchanges into two categories on the basis of symmetry is rather an indirect means of classification as the symmetry of an interchange is seen to be dependent upon two factors; (1) the polarity of the chromosomes involved, and (2) the manner in which the chromatid parts are exchanged. Chromosome polarity is probably a consequence of the anaphase movement of the chromosomes at mitosis, as the centromeric regions of the chromosomes pass first to the poles so that the centromeres lie closely grouped together at the later stages of mitosis. If little relative displacement occurs following the uncoiling and elongation of the chromosomes and during the succeeding interphase, then

TABLE 1

Relative frequencies of asymmetrical and symmetrical interchanges found by various authors

Material	No. of interchanges scored		Reference
	Symmetrical	Asymmetrical	
Tradescantia, pollen grain mitosis	26	66	SAX and MATHER (1939)
	182	402	SAX (1940)
	1613	1424	CATCHESIDE, LEA and THODAY (1946)
Tradescantia, pollen tube mitosis	49	46	NEWCOMBE (1942)
	15	70	SWANSON (1942)
	53	60	SWANSON (1942)
	12	9	CATCHESIDE and LEA (1943)
Chortophaga neuroblasts	5	9	CARLSON (1941)
Vicia faba root tips			THODAY (1948)
X rays	171	182	
α rays	151	190	

at the early prophase stage of the following mitosis the chromosomes should still show polarity. Evidence that no extensive chromosome movement occurs during interphase is given by the classical observations of BOVERI (1909) on Ascaris embryos and BELAR (1929) on the staminal hair cells of Tradescantia; these authors found that the chromosomes at prophase maintained roughly the same relative positions that they were observed in at the late stages of the previous mitosis. SAX (1940) found that the interphase chromosomes in Tradescantia microspores were strongly polarized as unequal interchanges induced by radiation were rare, and over 80 percent of the interchanges were found to involve exchange between chromosomes or chromatids at corresponding loci in respect of the distance from the centromeres. A similar conclusion is arrived at for the chromosomes in root-tip cells of *Vicia faba*. In an early experiment, interchanges were classified on the basis of symmetry and completeness and also with regard to the distance separating the centromeres, equal or roughly equal interchanges were defined as those in which the two centromeres were within a given axial distance from one another, the distance being arbitrarily defined in this instance as being about one quarter of the length of an acrocentric metaphase chromosome (about 2μ). Of 93 interchanges scored in this experiment 79 (i.e., 85 percent) were found to involve polarized chromosomes and 63 (70 percent) polarized chromosomes whose centromeres were close together, i.e., equal or roughly equal interchanges.

The second factor which determines the symmetry of an interchange relates to the actual mechanics of the exchange event itself. An exchange between chromatids of two chromosomes can occur in two ways as shown in Figure 1. The exchange can be similar to the type of exchange which occurs normally at meiosis, which we may call the X type exchange (Figure 1b), or the exchange may be of the form shown in Figure 1a which we may simply call the U type

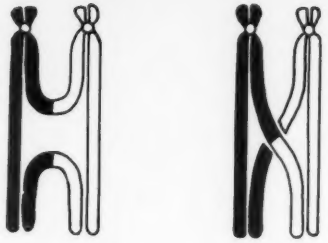

FIGURE 1.—Diagram showing two interchanges as observed at metaphase illustrating (a) the U type exchange and (b) the X type exchange.

exchange. An X type of exchange between chromatids of polarized chromosomes will yield a symmetrical interchange, whereas a U type exchange will be asymmetrical because of the formation of a dicentric chromatid.

On the three criteria of polarity of the chromosomes—(polarized, P, and non-polarized, N) type of exchange (X or U), and completeness (complete, C, and incomplete, I)—we can classify chromatid interchanges into eight morphologically distinguishable types, and the two asymmetrical incomplete types (PUI and NXI) can be further subdivided into four groups (Figure 2). The interchanges scored in four separate experiments were classified into these eight categories and the results obtained are shown in Table 2.

CATCHESIDE, LEA and THODAY (1946) showed that the relative frequency of A to S chromatid interchanges and of C to I types, was independent of dose, dose-rate, and temperature. Similarly in the present experiments there was no detectable effect of dose on symmetry and completeness. However, before going on to an analysis of the results a further factor must be borne in mind, namely the question of whether the mechanical pressure exerted on the cells during the preparation of the squash influences the degree of completeness and the orientation of the interchanges. In a few of the cells in a well squashed root-tip preparation the satellites of the two metacentric chromosomes are sometimes broken off at the nucleolar constriction, due to the mechanical pressure exerted on the slide. There also appears to be a tendency for such mechanical breakage to occur at the points where achromatic lesions or "gaps" have been induced by the radiation, and such fractures are morphologically indistinguishable from true chromatid deletions induced by the radiation. The importance of such mechanical breaks in interchange associations is not known, but it is likely that a small proportion of the complete interchanges are scored as incomplete types, due to mechanical breakage occurring at the point where the exchange took place. Mechanical pressure could also influence the orientation of an interchange in that an X type exchange could be squashed into a U type or *vice versa;* for example a PXC interchange would be scored as NUC if on squashing the chromosomes were displaced in different directions or if one of the chromosomes was

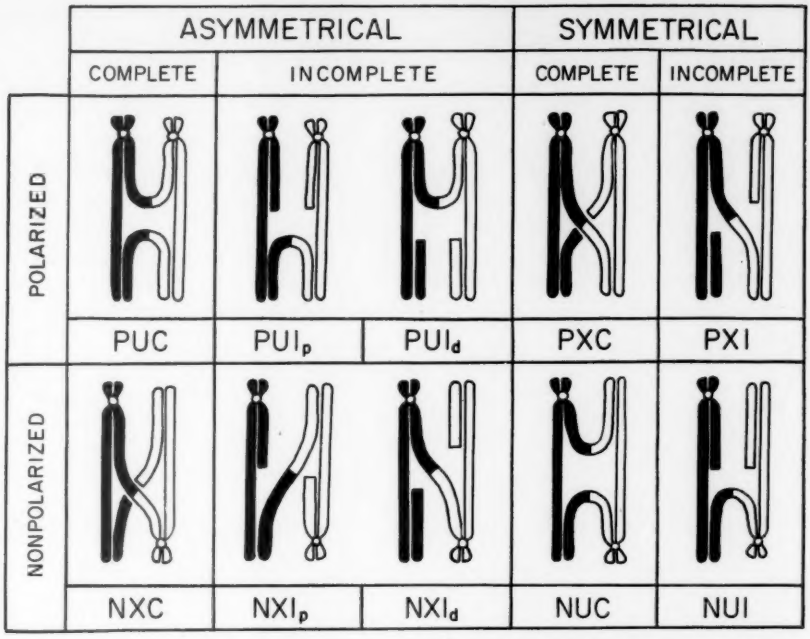

FIGURE 2.—Diagram showing the ten types of chromatid interchange P = polarized; N = nonpolarized; U and X are types of exchange; C = complete; I = incomplete; Ip = proximally incomplete; Id = distally incomplete.

TABLE 2

*Frequencies of the various interchange types in four experiments on diploid roots**

Interchange type	Asymmetrical				Symmetrical				
Expt.	PUC	PUI	NXC	NXI	NUC	NUI	PXC	PXI	Totals
1	121 (0.48)	35 (0.14)	2 (0.01)	2 (0.01)	15 (0.06)	1 (0.004)	40 (0.16)	34 (0.14)	250
2	74 (0.49)	27 (0.18)	1 (0.01)	1 (0.01)	9 (0.06)	3 (0.02)	23 (0.15)	12 (0.08)	150
3	80 (0.35)	35 (0.15)	9 (0.04)	4 (0.02)	12 (0.05)	8 (0.04)	48 (0.21)	32 (0.14)	228
4	50 (0.50)	10 (0.10)	1 (0.01)	1 (0.01)	13 (0.13)	5 (0.05)	15 (0.15)	5 (0.05)	100
Totals	325 (0.45)	107 (0.15)	13 (0.02)	8 (0.01)	49 (0.07)	17 (0.02)	126 (0.17)	83 (0.11)	728

* The figures in parentheses are the frequencies expressed as percentage values. The symbols P and N are for polarized and nonpolarized chromosomes, U and X are types of exchange, C and I are for complete and incomplete interchange types.

turned through 180°. It is likely that gross localized displacement of chromosomes due to squashing is an infrequent event, and this is evidenced by the fact that only 12 percent of the total interchanges scored were found to have nonpolarized chromosomes at metaphase (types NX and NU in Table 2), and also by the observation that the ratios PX:NU and NX:PU are dissimilar.

The results obtained in the four experiments shown in Table 2 have been subjected to analysis in order to examine the main effects—polarity, type of exchange and completeness—and to see if there were any interactions between these effects. The main effects were subjected to χ^2 or variance ratio tests and all were found to differ significantly from a hypothetical 1:1 ratio; in addition there was a significant excess of asymmetrical over symmetrical interchange types (Table 3). Interactions were examined by fixing the main effects to be as observed, and the analyses were performed using partition of χ^2 (LANCASTER 1951) and the log likelihood ratio test (KULLBACK 1959). Both analyses showed that there was no effect of polarity on completeness (PC) or of polarity on the type of exchange (PU). However, the interaction between completeness and type of exchange (CU) was found to be significant at the five percent level: further examination showed that this interaction was largely concentrated in experiment 1, which was the first experiment in which interchange aberrations were scored, and an analysis of the data excluding the first experiment revealed no significant interactions.

The results thus indicate that a large degree of chromosome polarity is present in the interphase nucleus and that chromosome polarity does not influence the type of exchange. Of the two possible types of exchange that can occur between chromatids the chiasma or X type exchange occurs less frequently than the U type; because of this, coupled with the high frequency of polarized chromosomes, asymmetrical interchanges are more frequent than symmetrical types.

TABLE 3

Analyses of main effects for agreement with a hypothetical 1:1 ratio between the two possible classes for each effect

Effect	Observed ratio	χ^2	P
Polarity			
P:N	0.88 : 0.12	442	< 0.001
Exchange			
U:X	0.68 : 0.32	*	0.5–0.01
Completeness			
C:I	0.70 : 0.30	122	< 0.001
Symmetry			
A:S	0.62 : 0.38	44	< 0.001

* Because of heterogeneity between experiments for this effect the analysis was performed using a variance ratio test (F_1 and $F_9 = 20.7$).

The experiment with roots treated with colchicine

Following upon the finding of a high degree of polarity of the chromosomes and the inference that no gross chromosome movement occurred during interphase, an experiment using a colchicine technique was set up to give further information on these points and also on the effect of polyploidy on the frequency of interchange aberrations. The technical details of the colchicine experiment have been described under the heading of Experimental Methods and the rationale behind the experiment was as follows. Roots were subjected to colchicine for 3½ hours and then returned to water so that cells in the early stages of mitosis and the later stages of interphase at the time of cholchicine treatment would not pass through a normal anaphase stage and would become polyploid as a result of spindle inhibition, while the cells in the earlier stages of interphase and in the postmetaphase stages of mitosis at the commencement of treatment would remain diploid. In this way, following the colchicine treatment, the meristematic regions of the roots would consist of a mixture of diploid and tetraploid cells and as the mitotic cycle in these root tip cells at 19°C takes about 18 to 24 hrs, some of these tetraploid cells would be in mitosis about a day after the colchicine treatment (EVANS, NEARY and TONKINSON 1957, 1959). Due to the natural variation in the rate of development of cells through the mitotic cycle, at the time of irradiation (21½ hours after completion of the colchicine treatment) diploid and tetraploid cells in approximately the same stage in development were being irradiated and the chromatid aberrations in such cells could be observed at metaphase in roots which were fixed between four and six hours after irradiation.

The results obtained from the colchicine experiment are described under three separate headings.

1. *The effect of colchicine treatment on chromosome polarity:* The frequencies of the various types of interchange were determined from scores obtained from 2,400 diploid and 1,800 tetraploid cells. The interchanges were categorized as described in the first part of this paper, and the results obtained are given in Table 4.

It was anticipated that in the tetraploid cells, which would have passed through two interphase periods in the absence of the polarizing influence of an intermediary anaphase, there might be a reduction in the amount of polarity and consequently in the frequency of A type interchanges. The results in Table 4 show that there is in fact little difference between the frequency of polarized interchanges in diploid and tetraploid cells (78 percent and 84 percent, respectively) and that the frequencies are similar to those obtained in the four experiments on diploid roots reported in the first part of this paper. The results thus suggest that there is no large scale chromosome movement during "c"-mitosis and that a high degree of chromosome polarity might still be in evidence over a number of mitotic cycles even in the absence of mitotic spindles.

To test for possible differences in the relative frequencies between diploid and

TABLE 4

*Frequencies of the various interchange types in diploid and tetraploid cells in roots subjected to colchicine**

Interchange type	Asymmetrical				Symmetrical				
Ploidy	PUC	PUI	NXC	NXI	NUC	NUI	PXC	PXI	Totals
2n	250 (0.33)	132 (0.17)	55 (0.07)	18 (0.02)	78 (0.10)	19 (0.03)	164 (0.22)	48 (0.06)	764
4n	361 (0.33)	184 (0.17)	59 (0.05)	24 (0.02)	79 (0.07)	27 (0.02)	265 (0.24)	111 (0.10)	1,110
Totals	611 (0.33)	316 (0.17)	114 (0.06)	42 (0.02)	157 (0.08)	46 (0.03)	429 (0.23)	159 (0.08)	1,874

* The figures in parentheses are the frequencies expressed as percentage values. The symbols P and N are for polarized and nonpolarized chromosomes, U and X are types of exchange, C and I are for complete and incomplete interchange types.

tetraploid cells the data in Table 4 were analyzed employing the two methods used for the data on diploid roots—the log likelihood ratio test (KULLBACK 1959) and partition of χ^2 (LANCASTER 1951)—and a significant difference between diploid and tetraploid data was suggested ($P_7 = 0.02$). On further inspection it was found that this was due to interactions in the diploid data which were mainly the result of the relatively lower frequency of PXI type interchanges; omission of the PXI class from the analysis resulted in homogeneity and no significant difference between diploid and tetraploid cells, ($P_6 = > 0.2$). Averaging the total data gives the frequency of polarized interchanges as 81 percent, U type exchanges 61 percent, completeness 70 percent, and asymmetrical interchanges as 58 percent. These values are similar to those obtained from the experiments on diploid roots (Table 3).

2. *The frequency of interchange between chromatids of homologous chromosomes in diploid and tetraploid cells:* The chromosome complement of diploid cells of *V. faba* is made up of one pair of easily distinguishable metacentric, or M, chromosomes and five pairs of acrocentric chromosomes, the S chromosomes (Figure 3). The two arms of an M chromosome are dissimilar both in length and in morphology and are easily distinguished, the shorter arm (M_2, as opposed to the long arm M_1) having a secondary constriction at the nucleolar organizing region. Of the five pairs of S chromosomes three (Sa, Sd and Se) are easily separated on the basis of the length of the long and short arms, and the remaining two chromosomes, Sb and Sc are very similar. In the present paper the S chromosomes will generally be treated as one group, and details of the frequency of interchange between the various S chromosomes will be presented in a separate paper.

A simple estimate of the frequency of interchange between homologous chromosomes or chromosome arms can be obtained by determining the frequency of M_1 to M_1, M_2 to M_2, and S to S interchanges. Now in cells which have been allowed to proceed through a c-mitosis the chromatids of a given chromo-

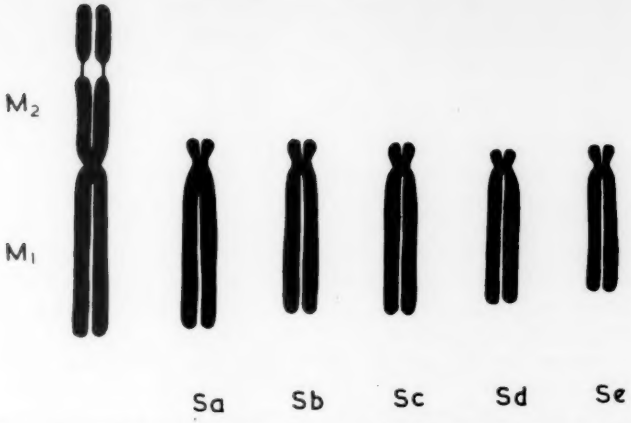

FIGURE 3.—Diagrammatic representation of the haploid chromosome set of *Vicia faba* (Sutton's Prolific Longpod).

TABLE 5

*The frequencies of interchange between similar and different chromosomes in diploid and tetraploid cells**

Fixation time (hrs)	Interchange type: Ploidy	S to S	M to S		M to M			Total interchanges
			M_1 to S	M_2 to S	M_1 to M_1	M_1 to M_2	M_2 to M_2	
4		61	23	9	2	3	2	100
5	2n	57	21	16	2	3	1	100
6		64	12	20	1	2	1	100
Total		182	56	45	5	8	4	300
		(0.607)	(0.187)	(0.150)	(0.017)	(0.027)	(0.013)	
4		61	17	10	6	4	2	100
5	4n	67	14	11	2	3	3	100
6		64	13	15	3	3	2	100
Total		192	44	36	11	10	7	300
		(0.640)	(0.147)	(0.120)	(0.037)	(0.033)	(0.023)	

* Percentage values are given in parentheses.

some will not be separated to opposite poles of the cell because of the absence of a spindle: thus, if there is little chromosome movement following the separation of the chromatids and throughout the following interphase, such cells, which will appear as tetraploids at the next mitosis, should show a higher frequency of interchange between homologous chromosomes than their diploid counterparts. The possibility of a difference in the relative frequency of interchange between homologous chromosomes in diploid and tetraploid cells was investigated, and 300 interchanges in diploid cells and 300 in tetraploid cells were scored and classified into the six possible groups as shown in Table 5.

The results in Table 5 are interesting in two respects. Firstly the relative frequency of the interchange classes S to S, M_1 to M_1 and M_2 to M_2 is seen to be increased in the tetraploid cells by roughly six percent. Now the small increase in S to S type interchanges is not very revealing as we have no information on how many of the S to S type interchanges have taken place between truly homologous chromosomes. On the other hand, although there are relatively fewer interchanges involving M to M chromosome, exchanges between homologous chromosome arms have been distinguished and are seen to be twice as frequent in tetraploid cells as opposed to the diploid cells. Although a small increase in the frequency of homologous interchanges is to be expected on statistical grounds (EVANS and BIGGER 1961), the observed doubling of the frequency of homologous interchanges in tetraploid cells is additional evidence indicating that little chromosome movement occurs during interphase.

The second interesting piece of information from these data concerns the question of the relation between chromosome length and the probability of a chromosome taking part in an interchange. We have measured the lengths of the metaphase chromosome in 12 cells and the average values, converted to microns, are $M_1 = 8.7$, $M_2 = 8.0$, and the five S chromosomes give a total of 36.1; the ratio, in terms of length, of the metacentric to acrocentric chromosomes is thus M:S = 1:2.16. Now if the probability of a chromatid undergoing interchange is a simple function of chromosome length, then the ratio of M:S chromosomes which have undergone exchange should conform to the ratio of 1:2.16. It may be seen from the data in Table 5 that the results do not fit this expectation ($x^2 = 45.4$, $P = < 0.001$), for the observed ratio in which these chromosomes take part in interchange is 1:3.44 for both diploid and tetraploid cells, i.e., the S chromosomes undergo interchange more frequently than is expected on the basis of metaphase chromosome length. The relative frequency of exchange by the arms of the M chromosome, i.e., M_1:M_2, with themselves or with the S chromosomes (but excluding the interarm M_1 to M_2 type interchanges), should on the basis of relative lengths be M_1:M_2 = 1:0.92, the observed frequency with which these chromosomes undergo exchange (data from Table 5) is 1:0.78. There is thus an apparent tendency for the M_2 chromosome arm to undergo rather less exchange than is predicted, but this does not differ significantly from expectation ($x^2 = 1.57$, $P = 0.2$–0.3). The data as a whole suggests that the probability of a chromatid taking part in an interchange is not a simple function of its length at metaphase; further and more detailed experimental results on this point will be reported in the second paper in this series.

3. *The effect of polyploidy on the frequency of interchange and isochromatid aberrations:* The influence of polyploidy on chromosome radiosensitivity has been the subject of study by a number of authors (SAX and SWANSON 1941; FRÖIER, GUSTAFSON and TEDIN 1942; and SMITH 1943, 1946). BISHOP (1952) found that the chromosome aberration frequency, per cell, in microspores of the tetraploid *Tradescantia virginiana* (2n = 24) was, in general, twice the frequency produced by the same radiation dose in *T. paludosa* (2n = 12). Thus the aberration frequency per chromosome was the same in the two species, although other data

obtained from cells irradiated in the interphase prior to pollen tube mitosis indicated that the tetraploid cells of *T. virginiana* were significantly more than twice as sensitive than the diploid *T. paludosa*. The difficulties of comparing aberration frequencies in diploid and tetraploid cells of different species, or of cells irradiated at different stages in development, were eliminated when CONGER and JOHNSTON (1956) scored chromosome aberration frequencies in an irradiated *Tradescantia paludosa* flower bud, which on examination was found to consist of a mixed population of haploid and diploid microspores. CONGER's results showed that for both chromosome deletions and exchanges, the aberration frequency in diploid microspores was exactly twice the frequency in haploid cells.

In the present work the production of root chimaeras containing both diploid and tetraploid cells, and the irradiation of such cells at similar stages in development along the mitotic cycle, offered an excellent opportunity for examining the influence of polyploidy on radiosensitivity in a different species and using chromatid as opposed to chromosome aberrations as a measure of effect.

The frequencies of interchanges, obtained from the data scored for the relative frequencies of the various types of interchange (Table 4), are given in Table 6. An analysis of variance on the data showed that there was no effect of fixation time on interchange frequency ($VR = 0.004$, $P_{(2,18)}, = > 0.2$), but a highly significant difference existed between the frequencies in 2n and 4n cells ($VR = 35.3$, $P_{(1,18)}, = < 0.001$). The over-all mean frequency of interchanges in 2n cells was 0.318 ± 0.06, and in 4n cells 0.617 ± 0.07; interchanges are thus seen to be twice as frequent, on a per cell basis, in the tetraploid cells (χ^2 for a 2:1 ratio of 4n:2n $= 0.46$, $P = 0.5$). Expressing the data in terms of interchanges per chromosome, and treating the arms of the metacentric chromosomes as separate chromosomes, the frequency of interchange is the same in 2n as in 4n cells and is respectively 0.0227 ± 0.0044 and 0.0220 ± 0.0026 per chromosome ('t' between chromosomes in 2n and 4n cells $= 0.13$, $P_{18} = 0.9$).

TABLE 6

Frequency of chromatid interchanges in diploid and tetraploid cells

Fixation time (hrs)	Ploidy	Total no. of cells scored	No. of normal cells	No. of cells containing one or more interchanges				Total no. of interchanges scored	Mean interchange frequency per cell
				1	2	3	4		
4	2n	800	565	225	10	..	..	245	0.31 ± 0.04
	4n	600	250	295	43	10	2	419	0.70 ± 0.04
5	2n	800	577	215	8	..	..	231	0.29 ± 0.04
	4n	600	310	240	44	5	1	347	0.58 ± 0.04
6	2n	800	530	253	16	1	..	288	0.36 ± 0.02
	4n	600	303	257	33	7	..	344	0.58 ± 0.05
Totals	2n	2,400	1,672	693	34	1	..	764	0.318 ± 0.06
	4n	1,800	863	792	120	22	3	1,110	0.617 ± 0.07

In addition to scoring for interchanges the frequency of isochromatid breaks in 2n and 4n cells was also determined, and the results are presented in Table 7. As in the case of the interchange class, isochromatid breaks are twice as frequent in 4n cells as in 2n cells (χ^2 for a 2:1 ratio of 4n to 2n = 0.18, P = 0.7). However, one interesting difference between the frequencies of isochromatid and interchange aberrations is seen from a comparison of the relative distribution of these aberrations between cells. It may be seen (Table 8) that the relative frequencies of cells containing different numbers of isochromatid aberrations gives a good fit to a Poisson distribution, whereas the distribution of interchanges between cells, both for 2n and 4n types, is not Poisson.

DISCUSSION AND CONCLUSIONS

Chromosome polarity and chromosome movement during the mitotic cycle: The results from the experiments on diploid roots on the frequencies of the various interchange types have shown that about 88 percent of the interchanges observed at metaphase have occurred between polarized chromosomes. Although

TABLE 7

Frequency of isochromatid aberrations in diploid and tetraploid cells

Ploidy	Total no. of cells scored	No. of normal cells	No. of cells containing one or more isochromatid aberrations			Total no. of isochromatid aberrations	Mean frequency of isochromatid aberration per cell
			1	2	3		
2n	200	180	19	1	..	21	0.105 ± 0.023*
4n	200	160	34	5	1	47	0.235 ± 0.034*

* The standard errors have been determined assuming a Poisson variate.

TABLE 8

The frequencies of cells with interchanges and isochromatid breaks, and the expected frequencies assuming a Poisson distribution

Aberration	Ploidy	Distribution	Frequencies of cells with indicated no. of aberrations					χ^2
			0	1	2	3	4	
Interchange	2n	Observed	1672	693	34	1	..	78.9**
		Expected	1746	356	88	10	..	
	4n	Observed	863	792	120	22	3	105.7**
		Expected	971	599	185	38	7	
Isochromatid	2n	Observed	180	19	1	..	..	....
		Expected	180	20	.	.	..	
	4n	Observed	160	34	5	1	.	0.63
		Expected	158	37	5	.	.	

** Significant at the 0.1 percent level.

10–20 percent of the interchanges are between chromosomes which at metaphase, have centromeres aligned in opposite directions, this does not necessarily imply that these chromosomes were not polarized in interphase. An apparent non-polarity at metaphase of chromosomes involved in an interchange could be due to the exchange occurring between overlapping distal ends of chromosomes whose centromeres are polarized to one side of the spherical interphase nucleus. We can conclude therefore that at least 80–90 percent of the chromosomes are polarized during interphase and that this polarity is probably a consequence of the grouping of centromeres following their movement along the mitotic spindle at the anaphase stage. The fact that this polarity is maintained throughout interphase implies that in spite of chromosome elongation and uncoiling at the late stages of mitosis there is relatively little change in position of chromosomes due to movement during interphase.

The above conclusions are reinforced by two types of observation made on interchange frequencies in the diploid-tetraploid root chimaeras. Firstly the finding that the relative frequency of interchange between polarized chromosomes in diploid cells was similar to that observed in tetraploid cells produced by colchicine, indicates that there is little change in the orientation and relative positions of the chromosomes during 'c'-mitosis as well as throughout two successive interphase cycles. It is of interest to note that many of the different types of movement of metaphase chromosomes, as observed in living cells using cinematographic methods (BAJER 1958), are absent or very much reduced in the absence of a mitotic spindle in 'c'-mitotic cells (MOLE-BAJER 1958).

The second important result having a bearing on the amount of chromosome movement that occurs during interphase is the observation that the irradiation in late interphase of cells which were allowed to proceed into that interphase without a true anaphase separation of chromatids, results in an appreciable increase in the frequency of interchange between homologous chromosomes. As may be seen from Table 5, the relative frequency of homologous interchange between the arms of the metacentric M chromosomes is twice as great in the tetraploid cells as in their diploid counterparts. This result suggests that there is little lateral movement of the chromosomes during interphase, and in the light of the observations on the polarity of the chromosomes it would appear that, from a mechanical point of view, the interphase chromosome is a relatively static structure.

The symmetry and completeness of interchanges and the nature of the exchange event: Interchange aberrations result in an exchange of chromatid parts between chromosomes and are in this sense similar to the normally occurring process of chiasma formation at meiosis. Beyond this, the comparison between radiation (or chemically) induced interchange and chiasma formation appears to break down. Three major points of difference are that chiasmata are formed between identical loci of homologous chromosomes, are never incomplete, and are always of the X type and are thus symmetrical, although an exception to this latter point is seen for example in the formation of a dicentric chromatid following normal X type chiasma formation within an inversion loop. In spite of these obvious differences between chiasmata and mitotic interchanges, and the fact that

they are initiated or produced by different means and in different cells, it is possible that the basic mechanics of exchange could be the same for both events, and that the asymmetry and incompleteness of an interchange may be a consequence of the relative positions of the chromatids involved with respect of the points of damage produced by the radiation and may not be *entirely* a function of the radiation itself.

The finding that at metaphase there are seen to be two types of exchange, the U and the X type, might suggest that there are indeed two different methods of exchange which can take place at the points of damage. We have shown that there is a significant excess of the U type as opposed to the X type exchange and this coupled with the high degree of chromosome polarity results in an excess of asymmetrical interchanges, but there is no interaction between the type of exchange and the polarity of the chromosomes involved. An alternative explanation for the presence of two types of exchanges at metaphase is that the exchange is of one form only and that the appearance of both U and X types at metaphase is the result of differences in the arrangement of the chromatids involved at the time when the interchange took place. We can illustrate this point as follows: if a chiasma-like, i.e. X type, exchange occurred between chromatids of two chromosomes, and at the point of exchange both chromatids were coiled, or both uncoiled, then the interchange would appear as an X type at metaphase. If however, an X type exchange occurred between a coiled and an uncoiled region of the two chromatids, then at metaphase the interchange would be of the U type (Figure 4), but the dicentric and acentric fragment should in some cases be interlocked. Such interlocking has not been observed, so that the presence of two types of exchange at metaphase is probably not a consequence of the fact that the chromosomes involved are not truly paired, but rather is an indication that the exchange process following radiation may take one of two forms.

No association was found between chromosome polarity, or type of exchange,

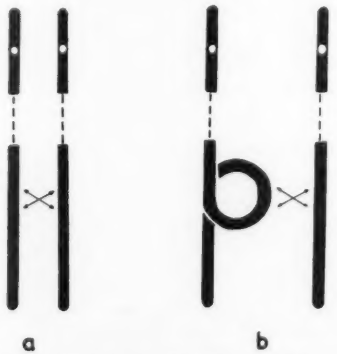

FIGURE 4.—The formation of interchanges which appear as X (a) and U (b) types at metaphase, although both are the result of X type exchanges during metaphase.

and incompleteness, although as reported by a number of authors (DARLINGTON and LA COUR 1945; CATCHESIDE, LEA and THODAY 1946) there is a higher frequency of incompleteness at the centromere, or proximal, side of the interchange. Of the 115 asymmetrical, incomplete, interchanges scored in diploid roots (PUI and NXI in Table 2) 0.57 were found to be incomplete on the proximal side of the interchange, cf. with 0.59 and 0.50 found in Tradescantia microspores by CATCHESIDE *et al.* (1946).

The amount of incompleteness is known to be influenced by the type of radiation used, for instance there may be more incompleteness with α radiation than with X or γ radiation (KOTVAL and GRAY 1947; FABERGÉ 1959), and this has led to the suggestion that incompleteness may be the result of severe damage to the chromatids at the points where the exchange will occur (LEA 1955). It is also possible that incompleteness may, in part, be a function of the distance separating the points which undergo exchange. If spatial separation is important, then in X or γ irradiated material there might be a possible association between the proportion of interchanges that are incomplete and the proportion that are two-hit although it should be noted that at present there is no evidence for correlation between dose-rate and incompleteness. In addition, it is important to point out that in squash preparations, some incompleteness may be due to mechanical breakage of a complete exchange due to the pressure used in the preparation of the slide.

The relation between chromosome length and interchange frequency: From the data in Table 5, which gives the frequency of interchanges involving S and M chromosomes, it has been shown that the frequency of interchange per chromosome does not appear to be directly proportional to the metaphase length of the chromosome. Although there are a number of instances where aberrations induced by radiation have been found to be distributed in a non random fashion along the length of the metaphase chromosome (see review by SPARROW 1951; EVANS and BIGGER 1961), the statement is frequently made, particularly with reference to radiation induced aberrations in Vicia (FORD 1949; DEUFEL 1951; REVELL 1953), that the distribution of such aberrations *between* chromosomes is in direct proportion to the metaphase length of those chromosomes. The present findings for interchange aberrations are at variance with this suggestion and following upon the present observations a detailed study of the distribution of interchange and isochromatid aberrations along the chromosomes was carried out, and the results and further discussion on this point will be presented in the second paper in this series.

The effect of polyploidy on chromatid aberration frequency: The frequency of both chromatid interchanges and isochromatid breaks in tetraploid cells was found to be twice the frequency observed in diploid cells. If primary breakage in the nucleus increases linearly with increasing dose, then it is to be expected that a doubling of the dose or of the chromosome number would both result in a doubling of the total number of breaks in the nucleus. However, as CONGER and JOHNSTON (1956) have pointed out, doubling the dose would double the fre-

quency of breakage per unit nuclear volume, but in the case of a doubled chromosome number if such an increase in chromosome number also results in a corresponding increase in nuclear volume, then the number of breaks per unit volume would remain constant.

A change in nuclear volume in tetraploid as against the diploid cells in Vicia would be of little importance with regard to intrachromosome aberrations and the finding that isochromatid aberrations are twice as frequent in 4n than in 2n cells is as might be expected. On the other hand, interchange aberrations involve the participation of two chromosomes and in Vicia, at the dose rates used in the present experiments, these aberrations are known to be predominantly two-hit and to increase as approximately the square of the dose (NEARY and EVANS 1958). There is some reason therefore for expecting that the interchange frequency in 4n cells might be four times and not twice the frequency found in 2n cells. In this respect the density of chromosomes per unit nuclear volume is of importance, and in the present experiments this density is approximately the same for both diploid and tetraploid cells as the 4n nuclei are roughly 2.2 times the volume of the 2n nuclei. In light of these considerations the observation that interchange frequency in 4n cells is only twice that in 2n cells "affords geometric evidence of the limitation of space over which chromosome breaks can interact to produce an exchange" (CONGER and JOHNSTON 1956).

The distribution of radiation-induced aberrations between cells is generally thought to be random and conform to a Poisson distribution (see discussion and review by LEA 1955). Recently, however, two important instances of deviation from Poisson distributions have been noted: JACKSON and BARBER (1958) found that the distribution of acentric fragments between root-tip cells of irradiated Allium seed showed a considerable degree of overdispersion in the data, and ATWOOD and WOLFF (1959) found that in Tradescantia microspores and in Vicia and Hordeum seed, the number of cells containing more than one chromosome interchange was always significantly smaller than that expected from a random distribution of aberrations, although other aberrations (isodiametric deletions) in the same cells did fit a Poisson distribution. In the present work the data from the colchicine experiment on the frequency-distribution of isochromatid and chromatid interchange aberrations is in many respects similar to that obtained by ATWOOD and WOLFF. The present results (Table 7) indicate that in both diploid and tetraploid cells the distribution of isochromatid aberrations is Poisson, whereas interchange aberrations do not fit a Poisson distribution, there being an excess of cells containing one interchange and a marked deficiency of cells containing more than one interchange. As ATWOOD and WOLF (1959) have pointed out, such a result indicates that interchanges are not induced randomly and that this is perhaps a consequence of the limitation in the number of sites in the nucleus where the chromatids of different chromosomes are sufficiently close together to undergo exchange. It is important to note that the distribution of interchanges in 2n and 4n cells is similar, as would be expected on the basis that chromosome density per unit volume is the same for both 2n and 4n nuclei.

SUMMARY

1. Radiation induced chromatid interchanges in root-tip cells of *Vicia faba* have been classified into eight, or ten, types on the basis of three criteria: (a) polarity of the chromosomes involved. (b) the manner in which the chromatid parts appear to have been exchanged, i.e. whether the exchange is of the U type or X type, and (c) whether the exchange is complete or incomplete.

2. Data from four experiments on interchanges in diploid roots indicated (a) that at least 80–90 percent of the chromosomes are polarized during interphase, and (b) that in about 70 percent of the interchanges observed at metaphase the exchange is of the U type. As a result of the high degree of chromosome polarity and high frequency of U type exchanges there is a significant excess of asymmetrical interchanges.

3. Although there were some slight indications of interaction between completeness and type of exchange, the results as a whole suggest that the three characters—polarity, type of exchange, and completeness—are independent of each other.

4. It is suggested that the presence of the U and X type interchanges at metaphase does not result from differences in the arrangement of the chromatids involved at the time of initiation of the exchange, but rather is an expression of two mechanically different types of exchange in interphase.

5. A study was made of the frequencies of chromatid interchanges and isochromatid aberrations in diploid and induced tetraploid cells of roots which were subjected to colchicine for 3½ hours, 21½ hours prior to irradiation.

6. The relative frequencies of the eight classes of interchanges were found to be similar for tetraploid and diploid cells. As 4n cells would have passed through two interphases without an intervening polarizing anaphase, the fact that chromosome polarity in 4n cells is as frequent as in 2n cells, together with the observation that there is a large increase in the frequency of interchange between homologous chromosomes in 4n cells, implies that little chromosome movement occurs during interphase and throughout 'c'-mitosis.

7. Observations on the relative frequencies with which the arms of the metacentric (M) chromosomes and the acrocentric (S) chromosomes undergo interchange, indicate that in both diploid and tetraploid cells the frequency of interchange per chromosome is not directly proportional to metaphase length of the chromosomes.

8. The frequencies of chromatid interchanges and isochromatid breaks in tetraploid cells was found to be twice the frequency observed in diploid cells, i.e. the frequency per chromosome is the same for 2n and 4n cells. The distribution of isochromatid breaks between cells, in both 2n and 4n cell types, fitted a Poisson distribution, but the distribution of interchanges was found to be non random.

ACKNOWLEDGMENT

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CHROMATID ABERRATIONS INDUCED BY GAMMA IRRADIATION II. NON RANDOMNESS IN THE DISTRIBUTION OF CHROMATID ABERRATIONS IN RELATION TO CHROMOSOME LENGTH IN VICIA FABA ROOT-TIP CELLS

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IN the experiments on *Vicia faba* root-tip cells reported in the first paper in this series (EVANS 1961), it was observed that the frequency with which the chromatids of various chromosomes, or chromosome arms, underwent interchange following irradiation was not in direct proportion to the metaphase length of these chromosomes. Taken as a group, the ten acrocentric chromosomes, compared with the two metacentric chromosomes, were found to have taken part in interchange more often than would have been expected on the basis of their relative lengths at metaphase, and it was thought that this deviation from expectation *between* chromosomes or chromosome groups might in fact be a consequence of a non random distribution of interchanges *within* the chromosomes.

The localization of aberrations induced by certain chemical treatments to particular regions of chromosomes is well known, and this is especially so in the case of chemical treatments of *Vicia faba* roots (FORD 1949; REVELL 1953; and MCLEISH 1953). A non random distribution of aberrations within chromosomes has also been reported for radiation-induced aberrations in other organisms (for some of the early papers see review by SPARROW 1951), and since the completion of the present work non randomness in the distribution of radiation-induced breaks has been reported between chromosomes of a variety of *Vicia faba* (REIGER and MICHAELIS 1959). In the present paper a study has been made of the frequency-distribution of radiation-induced isochromatid and interchange aberrations both within and between chromosomes of diploid cells. Additional information is also presented on the distribution and the frequency with which interchange occurs between homologous chromosomes in tetraploid cells, these cells being irradiated in the latter part of the interphase following their induction by colchicine treatment (EVANS 1961).

MATERIALS AND METHODS

Seedlings of *Vicia faba* (Suttons' Prolific Longpod) were cultured as described previously (EVANS, NEARY AND TONKINSON 1957), and the primary roots were exposed to 120 rads of Co^{60} gamma radiation given in 3.9 minutes under conditions of aeration. Fixations were made between 3½ and 5 hours after irradiation and cytological techniques were as described in EVANS (1961). For observations

on tetraploid cells the slides used in a previous experiment (EVANS 1961) were used.

Scoring was carried out at a magnification of about 1,200 diameters and to facilitate accurate mapping of the location of the points of exchange, an eyepiece graticule was used. The position of each point of interchange and, in the case of the isochromatid aberrations, intrachange was determined with reference to both the centromere and the distal end of the long arm of each chromosome. The chromosome complement, magnified some 15,000 ×, was accurately drawn on graph paper, and the length of each chromosome involved in an aberration was measured so that, after determining the appropriate correction factor, the points of exchange could be plotted on the graph paper. The reliability of the scoring system was checked by means of a number of parallel measurements where the locations of points of exchange were determined from photographs of aberrations photographed using the same optical system and enlarged to about 4,000 diameters; good agreement was found between both methods.

Using the above scoring technique 200 interchange aberrations were scored from ten slides made from diploid roots, so that points of exchange were located on 400 chromosomes: similarly 200 isochromatid aberrations were studied. The positions of 100 interchanges in tetraploid cells were mapped using the slides of the diploid-tetraploid root chimaeras (EVANS 1961).

RESULTS

The chromosome complement: The diploid chromosome complement of *Vicia faba* consists of five pairs of acrocentric chromosomes (the S chromosomes) and one pair of metacentric or M chromosomes (Figure 1). The two arms of the M chromosomes differ in length and are also distinguished by the fact that the shorter arm (M_2 as opposed to the longer arm M_1) has a well-defined secondary constriction at the point where the nucleolus is organized. In the variety used in the present work the longest and the two shortest of the S chromosomes (Sa, Sd and Se) are easily distinguished on the basis of their total lengths and the relative lengths of their long and short arms. Sb and Sc although easily picked out from the rest of the complement are difficult to distinguish from one another, except in the most favorable preparations, and for the purpose of plotting the distribution of aberrations these two chromosomes were treated as being identical. The relative lengths of the chromosomes and, in the case of the M chromosome, of the chromosome arms as determined from measurements made on 12 cells in metaphase are $M_1 = 8.7 \pm 0.5$ $M_2 = 8.0 \pm 0.4$, $Sa = 8.2 \pm 0.3$, $Sb = 7.7 \pm 0.4$, $Sc = 7.2 \pm 0.3$, $Sd = 6.8 \pm 0.3$ and $Se = 6.3 \pm 0.4$ microns: a diagrammatic illustration of the chromosome complement has been given earlier (EVANS 1961).

The distribution of interchange and isochromatid aberrations: To facilitate statistical analysis and to reduce errors inherent in the mapping technique each chromosome was divided up into eight equal segments, the M_1 and M_2 arms of the M chromosome being treated as separate chromosomes. As the metaphase chromosomes differ slightly in length, the size of the units into which each

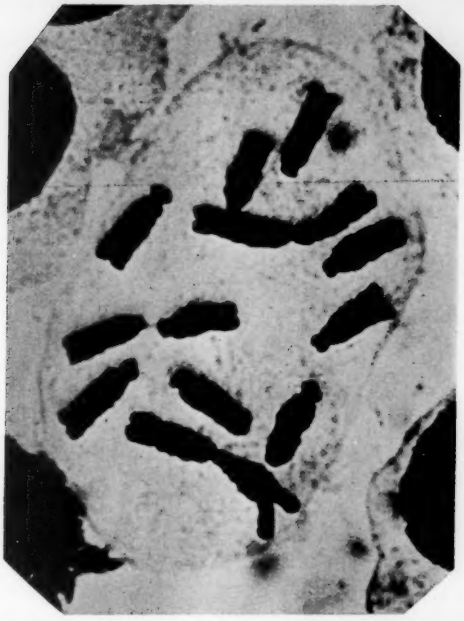

FIGURE 1.—Metaphase cell showing the normal diploid chromosome complement of *Vicia faba*. Note the five pairs of acrocentric S chromosomes and the two satellited metacentric M chromosomes (× 2,000).

chromosome is divided differs between chromosomes, although we may roughly say that each unit is approximately equivalent to one micron of a metaphase chromosome. The frequency per unit and the distribution of aberrations along the chromosomes, for the two types of cells studied, are shown in Figures 2 and 3. After completion of the scoring it seemed desirable to locate and map the position of the heterochromatin, and this was achieved from observations made on cells from roots kept at 3°C for four days (DARLINGTON and LA COUR 1940). The distribution of heterochromatin was found to be somewhat similar to that described by MCLEISH (1953) for a different variety of *Vicia faba* and is incorporated in the chromosome maps of Figures 2 and 3. It should be pointed out that the cold starved Feulgen-negative heterochromatin may not be synonymous with the Feulgen-positive heterochromatin of the interphase and mitotic nucleus: it is, however, usually adjacent to such positive heterochromatin and has therefore a similar distribution.

The results obtained on the distribution of interchanges and isochromatid breaks in 2n cells and of interchanges in 4n cells (see Figures 2 and 3) have been analyzed to test (a) whether the frequency with which a given chromosome takes part in an aberration is simply proportional to its metaphase length, and

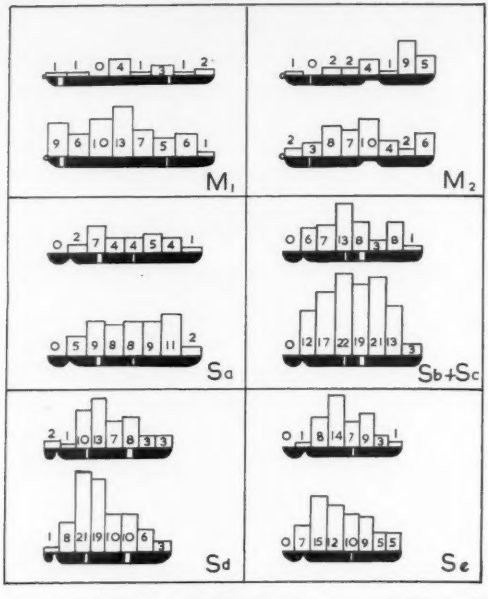

FIGURE 2.—Frequency distributions of 200 interchanges and 200 isochromatid breaks in diploid cells. Lower figures, interchanges: upper figures, isochromatid breaks. The positions of the heterochromatic regions are indicated by the white regions on the chromosomes.

TABLE 1

Frequencies with which the various chromosomes were involved in interchange in diploid cells

Chromosome	Metaphase length (μ)	Expected frequency on basis of length	Observed frequency	Contribution of χ^2 on the assumption of direct proportionality to chromosome length	Contribution of χ^2 on the assumption of a random distribution within the chromosome
M_1	8.7	65	57	1.23	12.75
M_2	8.0	61	42	5.92	11.71
Sa	8.2	62	53	1.31	15.98*
Sb + Sc	14.9	112	107	0.22	34.83***
Sd	6.8	51	78	14.29	36.05***
Se	6.3	48	63	4.69	19.41**
Totals	52.9	400	400	27.66***	.....

* Significance at the five percent level.
** Significance at the one percent level.
*** Significance at the 0.1 percent level.

(b) whether the aberrations are distributed randomly along the length of the chromosomes: the data were subjected to χ^2 tests and the results of the analyses are given in Tables 1, 2 and 3. In all cases it may be seen that the frequency of aberrations is not a simple function of chromosome length, and in many instances the distribution of aberrations within a chromosome is non random.

In Tables 4 and 5 the data on interchanges in 2n and 4n cells are presented to illustrate the frequency with which each chromosome underwent interchange with any other chromosome of the complement. It was pointed out earlier that it is difficult to make a distinction between chromosomes Sb and Sc and the data for these chromosomes are pooled, thus for the purpose of determining the fre-

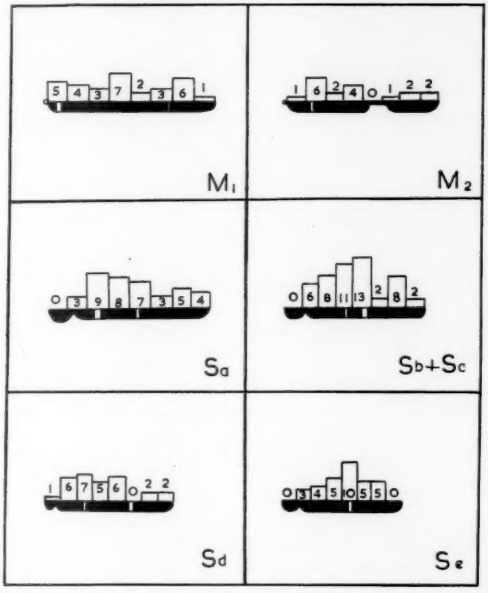

FIGURE 3.—Frequency distribution of 100 interchanges in tetraploid cells. The positions of heterochromatic regions are indicated by the white regions on the chromosomes.

TABLE 2

Frequencies with which the various chromosomes underwent isochromatid breakage in diploid cells

Chromosome	Metaphase length (μ)	Expected frequency on basis of length	Observed frequency	Contribution of χ^2 on the assumption of direct proportionality to chromosome length	Contribution of χ^2 on the assumption of a random distribution within the chromosome
M_1	8.7	33	13	12.12	7.31
M_2	8.0	30	24	1.20	20.00**
Sa	8.2	31	27	0.52	10.63
Sb + Sc	14.9	56	45	1.78	22.17**
Sd	6.8	26	47	16.96	21.94**
Se	6.3	24	43	15.04	31.61***
Totals	52.9	200	200	47.62***	....

** Significance at the one percent level.
*** Significance at the 0.1 percent level.

TABLE 3

Frequencies with which the various chromosomes were involved in interchange in tetraploid cells

Chromosome	Metaphase length (μ)	Expected frequency on basis of length	Observed frequency	Contribution of χ^2 on the assumption of direct proportionality to chromosome length	Contribution of χ^2 on the assumption of a random distribution within the chromosome
M_1	8.7	33	31	0.12	7.45
M_2	8.0	30	18	4.80	11.33
Sa	8.2	31	39	2.06	12.90
Sb + Sc	14.9	56	50	0.64	23.92**
Sd	6.8	26	29	0.35	13.76
Se	6.3	24	33	3.38	18.15**
Totals	52.9	200	200	11.35*	....

* Significance at the five percent level.
** Significance at the one percent level.

TABLE 4

Frequencies of interchange between homologous and non-homologous chromosomes in diploid cells

Chromosome	M_1	M_2	Sa	Sb+Sc	Sd	Se	Total chromosomes involved
M_1	*6*	5	12	9	16	9	57
M_2	5	*4*	5	13	4	11	42
Sa	12	5	*4*	12	13	7	53
Sb + Sc	9	13	12	36	22	15	107
Sd	16	4	13	22	*6*	17	78
Se	9	11	7	15	17	*4*	63
Total chromosomes involved	57	42	53	107	78	63	400

TABLE 5

Frequencies of interchange between homologous and non-homologous chromosomes in tetraploid cells

Chromosome	M_1	M_2	Sa	Sb+Sc	Sd	Se	Total chromosomes involved
M_1	*8*	4	8	4	5	2	31
M_2	4	*4*	1	3	3	3	18
Sa	8	1	*4*	16	7	3	39
Sb + Sc	4	3	16	12	4	11	50
Sd	5	3	7	4	*6*	4	29
Se	2	3	3	11	4	*10*	33
Total chromosomes involved	31	18	39	50	29	33	200

quencies with which interchange occurs between homologous chromosomes, chromosomes Sb and Sc must be excluded from the analysis. It may be seen from Table 4 that of the 111 interchanges that occurred between the five chromosome types that are easily distinguished, 12 of these interchanges took place between homologous chromosomes, i.e. a frequency of 10.8 percent. The tetraploid interchange data in Table 5 shows a higher frequency of interchange between homologous chromosomes; of the 56 interchanges between the five chromosome types, 16 occurred between homologous chromosomes, i.e. a frequency of 28.6 percent. This difference between 2n and 4n cells is significant ($\chi^2 = 8.4$, $P = < 0.01$), homologous interchanges being almost three times more frequent in the 4n cells.

DISCUSSION AND CONCLUSIONS

The results obtained on the relation between chromosome length and interchange frequency confirm our earlier observations (EVANS 1961) that the frequency with which a chromosome undergoes interchange is not directly proportional to its metaphase length. Further, this lack of proportionality between metaphase length and aberration frequency is not confined to interchange aberrations but is also evident in the data for isochromatid aberrations.

Dividing the total chromosome complement into two groups, the M and the S chromosomes, the metaphase length ratio of these chromosomes is M:S = 1:2.16. Previously (EVANS 1961) it had been found that the frequency with which the M and the S chromosomes underwent interchange, in both 2n and 4n cells, was in the ratio of 1:3.44, a ratio which differed significantly from that expected on the basis of chromosome length. In the present work the observed ratios for interchanges are M:S = 1:3.04 (2n cells) and 1:3.08 (4n cells); for isochromatid aberrations the ratio is somewhat higher, M:S = 1:4.40. These values are similar to the ratios of 1:3.47 and 1:4.42 reported in a recent publication by REIGER and MICHAELIS (1959) for X-ray induced aberrations in *Vicia faba*, and all are significantly different from the ratio expected on the basis of chromosome length at metaphase.

Consideration of a more detailed breakdown of the distribution of aberrations between chromosomes (Tables 1, 2 and 3) reveals that in the case of interchange aberrations, in both diploid and tetraploid cells, although both arms of the M chromosome undergo exchange less often than expected on the basis of metaphase lengths, the M_2 or nucleolar chromosome arm shows a bigger deviation from expectation than the M_1 chromosome arm. Amongst the acrocentric chromosomes the bulk of the deviation from expectation is due to the two shortest chromosomes, Sd and Se, both of which participate in interchange far more frequently than expected.

A somewhat similar pattern of results is found for the isochromatid aberrations (Table 2). Both chromosome arms of the M chromosome undergo isochromatid intrachange less often than expected, although unlike the interchange data the M_1 arm shows a bigger deviation from expectation than the nucleolar arm. As found for interchanges, the bulk of the deviation from expectation

amongst the S chromosomes is due to chromosomes Sd and Se, both of which have almost twice as many isochromatid breaks as would be expected on the basis of their lengths at metaphase.

A lack of direct proportionality between chromosome length and aberration frequency could, but need not necessarily, be a consequence of a non random distribution of aberrations within chromosomes. It may be seen from the present data that for some of the chromosomes studied the distribution of aberrations within these chromosomes is indeed non random, and it is possible that this non randomness may be responsible for the nonproportionality between chromosome length and aberration frequency.

A non random distribution of radiation induced aberrations along the length of a chromosome was reported by SAX and MATHER (1939), who found that in X-irradiated Tradescantia microspores there were significantly more aberrations per unit length of chromosome near the centromere than in any other chromosome region. SWANSON (1942) also observed a non random distribution of breaks in Tradescantia pollen tubes, but in this case more aberrations were found near the ends of the chromosomes. The data on the distribution of viable structural changes in Drosophila, as observed in salivary gland preparations, reveals a significant excess of breaks localized in heterochromatic chromosome regions. However, it is suggested that the relative frequency with which breaks are distributed between the euchromatin and heterochromatin regions approximated to the relative lengths of these regions in the mitotic cells, and that non randomness in the salivary glands may be due to differences in the relative compactness of these two types of chromatin in these cells (KAUFMANN 1946a, b).

Although the Drosophila results do not seem to indicate that heterochromatin has an important influence on the distribution of aberrations, in the last decade a number of investigators have reported a degree of localization of radiation-induced aberrations in the heterochromatic regions of the chromosomes of certain plant species. CAMARA, WAGNER and GARDE (1950) and GOTTSCHALK (1951) report that in Triticum and in tomato there is a tendency for localization of aberrations in or near heterochromatic regions of the chromosomes. Similar results have also been obtained by MOUTSCHEN and GOVAERTS (1953) on the sex chromosomes of *Cannabis sativa* and more recently by SIRE and NILAN (1959) on the chromosomes of Crepis.

The non randomness in the distribution of interchanges within chromosomes in the present data is mainly evident in the acrocentric or S chromosomes. The frequency-distribution of interchanges for the S chromosomes, as shown in Figures 2 and 3, is evidently not rectangular, and the principal deviation from randomness in all the S chromosomes is due to a marked deficiency of interchanges at the centromere and distal ends of the chromosomes and an excess of aberrations in the middle chromosome regions. A similar type of distribution is also evident for the isochromatid aberration data. In the M chromosome the distribution of interchanges along the chromosome arms is not significantly different from that expected on the hypothesis of a random distribution, although

the isochromatid data indicate a significant deviation from randomness due to an excess of aberrations in the satellite of the M_2 chromosome arm.

Although it is probable that with a larger scoring effort it might be shown that interchanges may also be non randomly distributed along the M chromosome arms, it is evident that their relative distribution within each arm is markedly different from that found for the S chromosomes. This difference between chromosomes is due to the fact that in the M chromosome, and more particularly the M_1 arm, there is no marked reduction in the frequency of interchange near the centromere and no excess in the middle regions of the chromosome arms. A study of the distribution of heterochromatin in the chromosomes suggests that the distribution of interchanges is in fact correlated with the distribution of heterochromatin (see Figures 2 and 3) and that the non random distribution of interchanges within the S chromosomes is due to an excess of interchange occurring in or near the heterochromatic regions which are located in the middle regions of the chromosomes. In the M chromosomes a considerable amount of heterochromatin is found near to and on either side of the centromere, and little is found in the midregions of the chromosome arms. This difference in the distribution of heterochromatin between M and S chromosomes parallels and would appear to be the cause of the difference in the distribution of interchanges within the two chromosome types.

The high frequency of interchange in or near the heterochromatic regions of the chromosomes may be responsible for the lack of direct proportionality between the metaphase length of a chromosome and the frequency with which it undergoes interchange. It is possible that the relative lengths of the chromosomes at metaphase differ from their relative lengths at interphase, and that the heterochromatic chromosome regions may be more compacted in the mitotic phases (cf. the Drosophila work discussed above). In spite of this possibility it is most unlikely that the frequency with which a heterochromatic chromosome undergoes interchange is directly proportional to its *interphase* length, for a considerable number of the interchanges take place between the euchromatic chromosome regions on either side of the heterochromatin. A more reasonable explanation for the partial localization of interchanges along the chromosomes is that there is an increased probability of contact between interphase chromosomes at or near the heterochromatic regions. The heterochromatin appears as Feulgen-positive dots in the interphase nucleus, and although as many as 20 discrete heterochromatic regions may be observed, these usually fuse to give a smaller number of larger chromocentres. It would appear therefore that the heterochromatic and adjacent euchromatic regions of a given chromosome are frequently in contact or in close proximity to other heterochromatic chromosomes. Such chromosome regions if they are damaged by radiation and if they are in contact, or in close proximity, would have a high probability of undergoing interchange.

The non random distribution of isochromatid aberrations within chromosomes is roughly of the same pattern as that found for interchanges, and although the former aberration involves chromatids of the same chromosome, it is still likely

that contact between homologous chromatids at heterochromatic regions is of importance in the distribution of such intrachanges. Although the excess of aberrations in the midregions of the chromosomes may be explicable in terms of the distribution of heterochromatin, this explanation does not satisfactorily account for the deficiency of aberrations at the chromosome ends. REIGER and MICHAELIS (1959) who also observed such a deficiency suggested that this emphasized the importance of the indirect action of X rays in the production of aberrations. We would not agree with their suggestion, but incline to the possibility that these deficiencies at the chromosome ends may be a consequence of the mechanical process of exchange. REVELL (1959) has suggested that chromatid aberrations involving single chromosomes, including the so-called simple breaks, are all the result of complete or incomplete exchanges between chromatids. Data obtained in our laboratory (NEARY and EVANS 1958; and unpublished work) has supported REVELL's observations and indicates that for intrachange aberrations the exchange may take place between sites of damage brought into close proximity due to looping of the chromosomes. On such a model, chromosome ends would have less opportunity for being in close proximity or in contact with other regions of the chromosome, as a result of looping, than the midchromosome regions, and this may of course be a possible explanation for the reduced aberration frequency at the chromosome ends.

The results have shown that the frequency of interchange or isochromatid aberrations involving either of the arms of the metacentric chromosomes, in both diploid and tetraploid cells, is less than is expected on the basis of their metaphase lengths (Tables 1, 2 and 3). In this connection it is of interest to note that for interchange aberrations the nucleolar or M_2 chromosome arm shows a bigger deviation from expectation than the M_1 chromosome arm, whereas the reverse is true for isochromatid aberrations. LA COUR (1953) has reported that in the garden Hyacinth, a variety which was trisomic for a nucleolar organizing chromosome showed a lower aberration frequency than a normal diploid variety following X-irradiation, and suggested that the presence of an extra nucleolar organizer and associated heterochromatin enhanced restitution. A similar suggestion was also made by MCLEISH (1955) from his study of radiation-induced aberrations in *Vicia faba* roots which had been treated with maleic hydrazide sometime prior to irradiation.

We have no evidence that the reduced aberration frequency on the M chromosome is due to an enhanced faculty for restitution on the part of this chromosome. We can, however, offer a rather different explanation for the reduced frequency of one particular type of aberration *viz.* the interchange aberration, more especially in conection with interchanges involving the M_2 or nucleolar chromosome arm. In the interphase nucleus the nucleoli occupy a considerable portion of the nuclear volume as each nucleolus is roughly about 7 μ in diameter. Clearly then the nucleoli attached to the M_2 chromosome arms must considerably reduce the probability of close proximity or of contact between the M_2 chromosome and any other chromosome of the complement. In about 47 percent of the interphase nuclei the two nucleoli are fused together, but such fusion does not appear to

place the homologous chromosomes in close proximity, for only few interchanges are found involving both M_2 chromosomes (Table 4). Spatial separation of the M_2 from the other chromosomes due to the nucleolus could thus account for the low frequency with which the M_2 arm is involved in interchange. Such nucleolar interference would have little direct effect on the intrachromosome aberrations, *viz.* isochromatid breaks, but might result in a compensatory rise in the frequency of such aberrations. We have been unable to find any published record of a buffering influence of the nucleolus on spontaneous or induced translocations involving nucleolar chromosomes, and it would be of interest to obtain further information from other organisms on this point.

In EVANS (1961) it was shown that in tetraploid cells which were produced by colchicine treatment and then irradiated in the following late interphase, the relative frequency of interchange between homologous arms of the M chromosomes was twice that found for diploid cells irradiated at the same stage in development. This result was interpreted as indicating that little chromosome movement occurred during interphase. In the present paper more detailed data (Tables 4 and 5) are available on this point, and these support our earlier work as the present data show that the frequency of interchange between homologous pairs of five types of chromosomes in the tetraploid cells is nearly three times the frequency found for diploid cells.

An increase in the frequency with which homologous chromosomes are involved in interchange in tetraploid compared with diploid cells is to be expected on statistical grounds, as shown below. Considering a single exchange between two chromatids in a diploid cell, then as there are 28 chromatids (treating the M_1 and M_2 arms as separate chromosomes) there are $\binom{28}{2} = 378$ possible combinations of pairs of chromatids. Of these, 14 such pairs or exchanges would be between homologous chromatids within a chromosome, i.e. isochromatid breaks, and of the remaining 364 true interchanges eight would be of the interarm (M_1 to M_2) type. Out of these 364 possible combinations of pairs 28, i.e. 7.69 percent, would be pairs of homologues. Thus, in diploid cells having one or more interchanges per cell, if all the chromatids had an equal probability of taking part in interchange with any other chromatid, 7.69 percent of the interchanges would be between homologous chromosomes. In tetraploid cells the number of combinations of pairs of chromatids, excluding 28 combinations which give isochromatid breaks, is $\binom{56}{2} - 28$ or 1,512, of which 168 or 11.11 percent are pairs of homologues. Thus assuming, as above, that all chromatids have an equal probability of undergoing interchange with any other chromatid, tetraploid cells would have about 50 percent more interchanges between homologous chromosomes than diploid cells. In actual fact we have shown that the chromosomes (chromatids) do not have equal probabilities for undergoing interchange, and we have therefore calculated the expected frequency of homologous interchanges in diploid and tetraploid cells using our observed values (from the diploid cell data in Table 1) for the probability of any chromosome undergoing interchange. The results obtained give values of 7.96 percent and 11.49 percent respectively, for the proportions of interchanges which should occur between homologous chromosomes in

2n and 4n cells. If we exclude chromosomes Sb and Sc from our calculations and deal only with the five clearly distinguishable chromosome types, the expected frequencies for interchange between homologous chromosomes in diploid and tetraploid cells is 11.62 percent and 16.47 percent to be compared with our observed values of 10.8 percent and 28.6 percent.

In the presentation of the results it was pointed out that the observed frequency of homologous interchange in tetraploid cells differed significantly from that found in diploid cells ($x^2 = 8.4$, $P = < 0.01$). However, if we now allow for the approximately 50 percent expected increase in tetraploid cells then our observed threefold increase is not significant at the five percent level ($x^2 = 2.52$, $P = > 0.1$), but the results taken together with our earlier observations (EVANS 1961) support the conclusion arrived at earlier that little chromosome movement occurs during mitotic interphase.

SUMMARY

1. A study of the frequencies with which the various chromosomes of *Vicia faba* root-tip cells were involved in interchange or isochromatid breakage following irradiation revealed that the frequency with which a given chromosome is involved in such aberrations is not proportional to its length at metaphase.

2. Whereas the distribution of points of exchange along the metacentric M chromosomes was approximately rectangular, the distributions for the acrocentric S chromosomes was non random, there being a significant excess of aberrations at the midchromosome regions and a deficiency at the centromere and distal ends.

3. It appeared that there was an association between the distribution of aberrations and the distribution of heterochromatin, there being a tendency for more aberrations to occur in or near heterochromatic regions. This association is believed to be due to an increased probability of heterochromatic or associated euchromatic chromosome regions to be in close proximity due to chromocentre formation in the interphase nucleus and is thought to be mainly responsible for the non random distribution of aberrations along the chromosomes and for the differences in aberration distribution within the M and S chromosomes.

4. The relatively low frequency of interchange involving the nucleolar or M_2 chromosome arm was not paralleled by a similar reduction in isochromatid aberration frequencies suggesting that this low frequency was due to the nucleolus acting as a physical barrier between the nucleolar and other chromosomes.

5. Data are presented on the frequencies of interchange between homologous chromosomes in diploid cells and in tetraploid cells which were irradiated in the interphase following their induction by colchicine treatment. The threefold increase in interchange between homologous chromosomes in tetraploid cells is in accord with the suggestion that little chromosome movement occurs during interphase.

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DIFFERENTIAL SENSITIVITY OF MOUSE SPERMATOGENESIS TO ALKYLATING AGENTS

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VARIOUS alkylating agents have long been used for inducing mutations and chromosome breaks in some plants and animals. Effects on Drosophila spermatogenesis have been investigated by AUERBACH (1940, 1949, 1958), especially with mustard gas, and FAHMY and FAHMY (1953, 1956) used various compounds. All types of mutations have so far been obtained—dominant lethals and semilethals, recessive lethals, visible viable mutations, and chromosome breaks.

Such intensive investigations have not been achieved in the mouse, in which the study of mutagenesis with radiomimetic compounds is just commencing. BATEMAN (1958) recorded dominant lethals and found some analogy in the distribution of breaks per egg between X rays and a polyfunctional imine, Triethylenemelamine (TEM). CATTANACH and EDWARDS (1958) found that TEM reduces the fertility of the male mouse.

The present work deals with a preliminary study of chromosome breaks observable at various times after treatment of spermatogenic cells of the mouse with alkylating agents. Some chemical tests *in vitro* and some experiments in plants suggested that polyfunctional compounds are more active than monofunctional ones. However, this idea is not universally accepted. For this experiment, in spite of this possible discrepancy, three polyfunctional compounds belonging to three quite different chemical classes were arbitrarily selected. Comparison with monofunctional chemicals will be reserved for future investigations.

MATERIALS AND METHODS

Myleran (Busulfan) (1,4-dimethanesulfonoxybutane): This substance has been used frequently in hematology as an antileukemic agent. Mutagenic activity of alkylmethanesulfonates was tested in Drosophila by FAHMY and FAHMY (1956). Some special properties of Myleran were reported by MOUTSCHEN and MOUTSCHEN-DAHMEN (1958).

Triethylenemelamine (2,4,6-triethyleneimino-1,3,5-triazine—TEM): Mutagenic and carcinogenic activity of this compound was tested by FAHMY and FAHMY (1954, 1955a,b). It has also been used in plant experiments.

Diepoxybutane (1,2,3,4-butadiene dioxide): This drug was tested in the plant

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kingdom, where REVELL (1953) carried out a cytological analysis of the induced lesions in *Vicia faba*. FAHMY and FAHMY (1953) also tested it for mutagenic and carcinogenic activity in Drosophila. Diepoxybutane also has been used for induction of gene mutations in Neurospora by KØLMARK (1953); KØLMARK and GILES (1953); KØLMARK and WESTERGAARD (1953); WESTERGAARD (1957).

Hybrid 101 × C3H mice were injected intraperitoneally when 5 to 7 months old and then killed at different times after treatment. After immersion for ½ hour in ½ isotonic NaCl solution, testes were fixed in acetic acid-alcohol (1:3) for 1 to 2 hours. They were then stained *in toto* with Schiff-Feulgen and observed after squashing.

The use of anaphase fragments and bridges for estimating damage caused by alkylating agents probably is not the best in absolute values, but it avoids some possibilities of mistakes that exist when metaphase lesions are considered. For instance, in metaphase the early separation of the heterochromosomes makes it difficult to distinguish these structures from fragments.

RESULTS

A. *Sensitivity of different stages of spermatogenesis:* In a first set of experiments, the following doses of chemical were used: diepoxybutane, 5 mg/kg; TEM, 6 mg/kg; Myleran, 16 mg/kg. Animals were killed at 20 hours and 2, 5, 10, and 20 days after injection. A few animals were killed at 3, 4, 7, and 30 days. Chromosome breaks estimated by anaphase fragments are plotted in Figure 1A, which shows that (1) the sensitivity of various stages is different for the three compounds; and (2) for Myleran, there are two peaks of sensitivity. The sensitivity diminishes from a stage presumably close to the resting stage of spermatocyte I to the middle of pachytene and then increases to another maximum. The peak at 1 to 2 days, according to OAKBERG's estimate (1956) of duration of spermatogenesis, must be near the end of pachytene or the beginning of diplotene. It has not been possible to determine the sensitivity of spermatogonial cells to chromosome breakage since these cells are killed by TEM and Myleran. Moreover, in such an attempt, we should have to compare the sensitivity of mitotic and

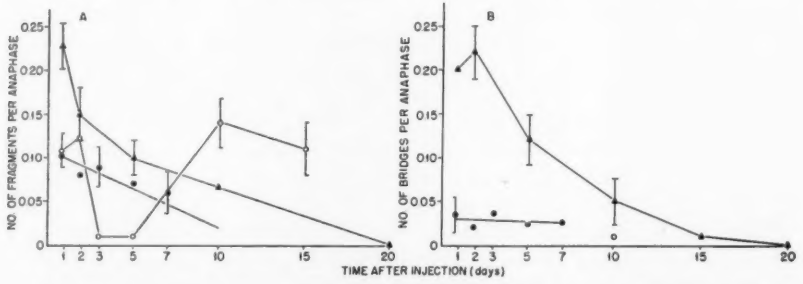

FIGURE 1.—Frequency of fragments and number of bridges in anaphase I after different times with diepoxybutane (▲), TEM (●), and Myleran (○).

meiotic divisions, which are not strictly comparable. Macroscopically observed, testicular atrophy occurs about 15 days after Myleran injection. (3) For TEM, there was testicular degeneration at 10 to 15 days. No sensitivity of early spermatogenesis to this chemical could be ascribed. (4) For diepoxybutane, no testicular degeneration was observed after 20 or 30 days or even longer times up to 50 days. For this compound, only one peak was found, which must be situated around diplotene; it corresponds to the peak for Myleran at day 1, but differs from Myleran in that a significant decrease in fragments but an increase in bridges occurs by day 2.

Although some fragments were scored in spermatogonial anaphases, the intensity of this damage was not severe enough to induce testicular degeneration. It is possible that DNA synthesis occurs at the time when a second peak has been found for Myleran, i.e., before leptotene, but it is unlikely that DNA synthesis is involved in the day-1 peak. Thus no conspicuous relation between peaks of sensitivity and the presumed time of DNA synthesis could be found. The difficulty of a possible disturbance of duration of spermatogenetic period by the poisons could not be avoided. These disturbances, which do not occur or at most are slight with X rays (EDWARDS and SIRLIN 1958; OAKBERG and DIMINNO 1960), would necessarily lead to difficulties in identification of cell stages at time of treatment. If they occur, the exact intensity of this kind of inhibiting effect on meiotic processes would have to be known. Presumably, the amount of inhibition could be different not only from one compound to another but also from one dose to another. In this first set of experiments, however, the injected amounts of drugs were not very toxic for the animal, especially Myleran, and we therefore think that the conclusions have not been seriously affected. A somewhat similar experiment was carried out with X rays in order to obtain a reference scale for estimating the intensity of damages induced with toxic substances.

B. *Relative efficiencies of chemicals and X rays:* In this set of experiments, as in the previous one, the average number of anaphase fragments was taken as the criterion for estimating the damage. These cells were scored 20 hours after injection since, as shown in Section A, at this time a peak of highest sensitivity for the three chemicals was obtained. The data are pooled in Figure 2A and show the results. (1) For the three chemicals, the fragment frequency increased with increasing dose. According to the restrictions made in Section A, after 20 hours the intensity of a possible meiotic disturbance cannot be very high. (2) The increase with dose is higher for diepoxybutane than for TEM and higher for TEM than for Myleran. (3) It seems to be difficult to exceed a certain level of effect. For diepoxybutane, a plateau was rapidly reached and was not exceeded, owing to a strong killing effect on the animals. Myleran doses were limited in these experiments by the low solubility of the substance. (4) At higher doses, TEM gave more lesions than the other compounds, but at these doses its toxicity for the animals is so high that it eliminates all practical possibilities of mutation induction.

These experiments show that, in future investigations on induction of mutations in the mouse with radiomimetic compounds, we will always be subject to

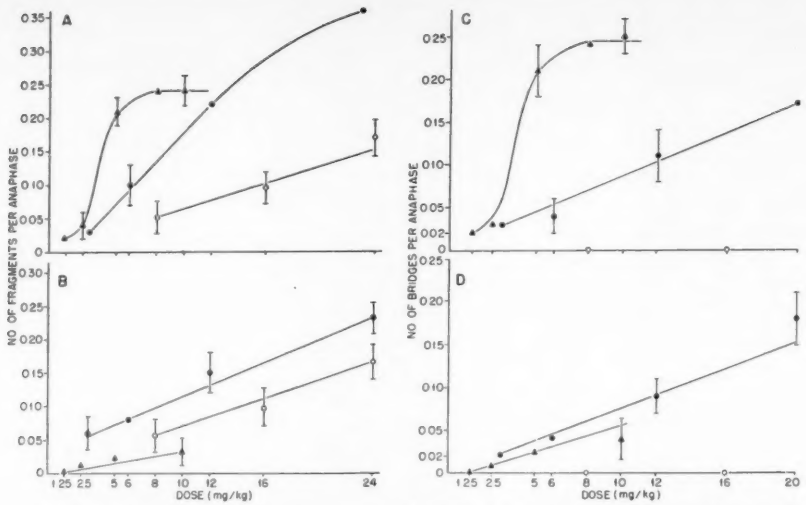

FIGURE 2.—Frequency of fragments in anaphase I (A, C, 300 cells) and anaphase II (B, ~600 cells; D, 300 cells) after varying doses of diepoxybutane (▲), TEM (●), and Myleran (○).

limiting factors, some of which are already well known from other work with plants and animals. We may be favored, however, by some special circumstances that result from the sensitivity of mammalian cells to specific compounds. If the activities of the chemicals are compared at the highest possible dose (say 10 mg/kg), diepoxybutane is the most efficient compound for breaking chromosomes, being about 1.5 times as efficient as TEM and four times as efficient as Myleran. Below these limits, the lower the dose, the more reduced are the respective efficiencies.

The following observations result from a comparison of the efficiency of the three compounds with that of X rays. (1) The efficiencies of the compounds on the second interphase were comparable with those observed for the first, but TEM was a little more efficient than diepoxybutane (Figure 3). The second meiotic division was chosen for this set of experiments because it is shorter. (2) The second meiotic cycle (including also probably late spermatocyte I stages) can be considered somewhat more sensitive to the drugs than the first one. This situation is similar to that obtained for ionizing radiations (OAKBERG and DIMINNO 1960).

The results summarized in Figure 3 show that the presumed sensitivity of the second interphase to toxic chemicals is not very great as compared with the sensitivity to radiations. This comparison is limited, however, to doses of chemicals low enough not to be toxic to the animals. This comparison does not afford all possibilities and therefore has no absolute value. Only the immediate numbers of lesions scored in anaphase II were compared. In fact, it is known that, with toxic substances, some delayed effects can exist that this scoring cannot take into

account. This method, therefore, probably underestimates the real number of lesions.

C. *Elimination of damage:* Fragment elimination could be estimated by comparing the average number of fragments in anaphases I and II. Two sets of experiments were performed. The first one represents a comparison, 20 hours after injection, of the effects of increasing the dose. In the second, the effects of stabilizing the dose while increasing the intervals were studied. Since a delayed effect can exist for chemicals, however, the present data measure not only the elimination of damage but also a combined action of elimination and new genesis. Elimination includes selective processes, nonvisible fragments at the poles, and probably early cell degeneration. The new genesis includes delayed appearance of lesions not visible before and also presumably subchromatidic lesions. The elimination of fragments is quite different for the three chemicals. Figures 2A and B summarize the results: (1) Elimination is higher for diepoxybutane than for TEM or Myleran. (2) For Myleran, there are no significant differences between the counts in anaphases I and II. This means that, with this compound, new genesis can be higher or elimination lower than for the other compounds, or both. This result agrees fairly well with some previous data in barley and the broad bean (MOUTSCHEN and MOUTSCHEN-DAHMEN 1958). (3) For diepoxybutane and TEM, the higher the dose, the more efficient is the elimination of fragments.

D. *Rejoining ability of broken ends:* In the previous experiments, the number of anaphase fragments was taken as the criterion for estimating the damaging effect of radiomimetic compounds. In the course of these experiments, it was also

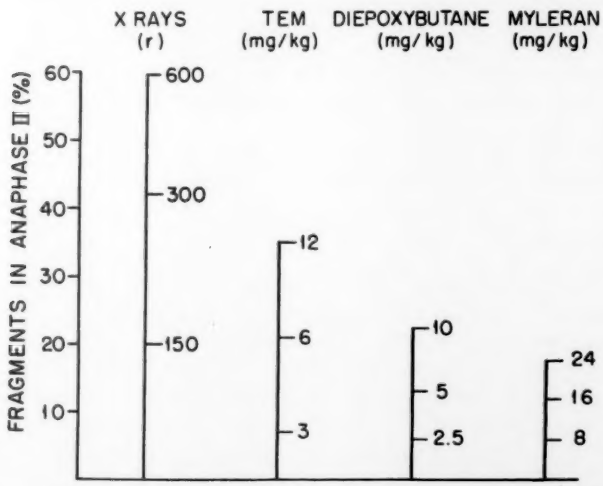

FIGURE 3.—Comparison of the relative efficiencies of X-rays and chemicals ten hours after treatment (300 anaphases).

possible to score the anaphase bridges and then, in some cases, to estimate the rejoining ability. The following findings came from the experiments.

(1) For diepoxybutane, if bridges are scored at different intervals after injection, as was done for fragments, the shapes of the curves are almost the same. (2) For TEM, there are comparatively fewer bridges. (3) For Myleran, the situation is obviously quite different. With this chemical, no bridges were observed until 10 days after injection. At all times, the number of observed bridges remains significantly lower than for the two other compounds; it may not be significantly higher than in the control. This peculiar situation is somewhat comparable to that described in barley and in the broad bean (MOUTSCHEN and MOUTSCHEN-DAHMEN 1958). (4) With both TEM and diepoxybutane, bridge frequency seems to parallel break frequency. The ratio of breaks to bridges is close to unity for diepoxybutane. Relatively, this substance seems to give the highest proportion of rejoinings. No bridges were observed with Myleran.

Figures 1B and 2C and D summarize the results. In Figure 1B, bridges were scored at several times after injection, as mentioned before. In Figures 2C and D, they were scored 20 hours after injection, respectively, in anaphases I and II. In anaphase II, instead of an increased number of bridges, a decrease can be noted. This suggests that, after one meiotic cycle, some broken bridges did not rejoin and could be due to some inherent properties of broken ends.

DISCUSSION

The stages yielding the most reliable data extend over the entire spermatocyte cycle, including maturation divisions. For both prespermatocyte and postspermatocyte stages, the sensitivity can be estimated only by some indirect means. For instance, a high sensitivity of spermatogonia to TEM and Myleran is indicated by testicular atrophy. In this respect, diepoxybutane acts quite differently. This could be a valuable property in future investigations, whereas the higher sensitivity of spermatogonial stages to TEM and Myleran could complicate future work with these substances. The efficacy of diepoxybutane in breaking chromosomes has been reported previously for other material. In Neurospora, mutagenic activity of the epoxides is very well known (JENSEN, KIRK, KØLMARK and WESTERGAARD 1951; KØLMARK and WESTERGAARD 1949; KØLMARK and GILES 1955).

With chemicals, a delayed effect in producing mutations and chromosome breaks has been definitely demonstrated. In plants, for instance, KIHLMAN (1952) found delayed breakage with a purine derivative, 8-ethoxycaffeine. With the same compound, it has been shown that some effects can be so delayed that they appear at the first generation after treatment (MOUTSCHEN and MOUTSCHEN-DAHMEN 1960). In Drosophila, treatment of spermatozoa with chemicals can show effects very late in ontogeny. Therefore, in these experimental results, a possible delayed effect must not be overlooked. It has been supposed that new genesis of breaks could occur in anaphase II as a result of subchromatidic breaks induced during the first spermatocytic stages. In this case, some attached frag-

ments should be seen at anaphase II. Since these fragments failed to appear, the occurrence of subchromatid breaks is very improbable. In plants, however, Myleran induced delayed chromosome breaks although few attached fragments were observed (MOUTSCHEN and MOUTSCHEN-DAHMEN 1958). Therefore, at least in Myleran experiments, other causes of delayed effects cannot be ruled out. This would explain the results of the comparisons between fragment numbers observed in anaphases I and II 20 hours after treatment.

At equal mortality, the efficiency of chemicals in production of chromosome breaks does not seem high compared with X rays. Here, too, a possible delayed effect of chemicals should not be disregarded for complete comparison. In plants, chromosome breaks induced with alkylating agents were not found to be randomly distributed but specifically localized at some "weak spots" (see FORD 1949, for mustard gas; REVELL 1953 for *NN*-di(2-chloroethyl)methylamine and some epoxides; OCKEY 1957, for some imino compounds; MOUTSCHEN and MOUTSCHEN-DAHMEN 1958, for Myleran). The same was found for Drosophila mutations by FAHMY and FAHMY (1957a), although the reliability of these last findings is not admitted by all investigators.

At the present time, there is no information on the qualitative aspects of chromosome breakage in the mouse. It would be worth extending these investigations to larger mammalian chromosomes.

Comparison of the sensitivity of various stages of spermatogenesis in the mouse with the organism in which it has been most investigated, i.e., Drosophila, could be valuable. In this material, AUERBACH (1958) showed that spermatozoa are very sensitive to mustard gas. A peak of higher sensitivity occurred in a stage before maturation of spermatozoa, probably spermatids. In Drosophila, too, the sensitivity of the same stages of spermatogenesis differs from one toxic compound to another. For instance, after 2-chloroethylmethanesulfonate, mutations were observed only in late broods or those arising from treated spermatogonia (FAHMY and FAHMY 1957b). The situation in the mouse might be comparable. It could be argued that, since chromosome breaks represent only a part of mutagenesis, these data have to be extended to all types of mutations. At the present, we could only speculate about the possible causes of differential sensitivity to the toxic compounds. It seems logical that a metabolically highly active tissue would be most sensitive. This explanation does not seem very plausible, however, when applied to the end period of pachytene and diplotene.

SUMMARY

F_1 males obtained by the cross of mouse strains 101 and C3H were injected with Myleran, diepoxybutane, and Triethylenemelamine (TEM). Chromosome fragments and bridges were scored in anaphases I and II and taken as a measure of the intensity of damage induced by the chemicals. Examinations were carried out at progressive intervals after injection of single doses of the three compounds and after 20 hours for progressively increased doses of the compounds.

A differential sensitivity of spermatogenic stages and some differences in ac-

tivity occurred. According to these differential responses, some future applications in genetics can be suggested.

At the same time, an attempt was made to estimate the elimination of lesions by comparing the numbers of lesions at anaphases I and II 20 hours after injection.

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INCOMPATIBILITY IN THE COMPLEX-HETEROZYGOTES OF OENOTHERA[1]

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MOST Euoenotheras which occur from the Rocky Mountains eastward to the Atlantic Coast are self-pollinating, true-breeding translocation or "complex"-heterozygotes. These forms have been classified according to their cytogenetical and morphological characteristics into several phylogenetic groups (CLELAND 1950) (Table 1). Evidence was presented earlier (STEINER 1956, 1957) that one of these, the biennis group 1, possesses an incompatibility allele system of the gametophytic type. The discovery of self incompatibility in the biennis group 1 adds another dimension to the unusual cytogenetic mechanism of the complex-heterozygotes and raises a number of problems of evolutionary as well as of genetic interest. Among these is the question as to whether such an incompatibility system is generally characteristic of the complex-heterozygotes, or whether it is unique to the biennis group 1. A survey of the other phylogenetic groups has been undertaken and brief reports of the preliminary results have been published elsewhere (STEINER and SCHULTZ 1958; STEINER 1959). In this paper the experimental data so far obtained are presented and evaluated in greater detail.

The essential features of the cytogenetic mechanism which characterizes the complex-heterozygote deserve a brief review in order to provide a background for our discussion. During the first meiotic division the 14 chromosomes of such an Oenothera become arranged in a circle; thus, each genome of seven chromosomes differs completely in its segmental arrangement from the other. At Anaphase I alternate members of the circle regularly go to the same pole, so that only two chromosomal combinations (complexes) result from meiosis. Although these forms are self-pollinating, homozygous combinations of the two complexes making up a complex-heterozygote do not occur among the offspring because of a balanced lethal system.

When a complex-heterozygote is outcrossed, it becomes apparent that one of the complexes is usually transmitted predominantly through the egg, while the other comes more readily through the pollen. The complex most often transmitted through the egg is called the alpha, while beta designates the complex passed on largely through the pollen. The alpha and beta complexes differ not only in the segmental arrangement of their chromosomes, but also with respect to the phenotypic characters which they produce. The phenotypes of the complexes which characterize each phylogenetic group are shown in Table 1.

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TABLE 1

Phylogenetic groups of complex-heterozygotes

Group	Geographical distribution	Phenotypic characteristics*
Strigosa	Rocky Mountain area eastward to Mississippi and beyond.	Alpha and beta complexes both produce strigosa phenotype showing narrow, gray-green leaves with appressed pubescence and a spreading habit.
Biennis 1	Ozarks east to the Atlantic Coast and throughout the Southeast.	Alpha complex produces a biennis phenotype with thin, dark green leaves, sparse pubescence; beta complex carries typical strigosa characters.
Biennis 2	Northeast United States southward to Virginia and North Carolina.	Alpha complex produces a strigosa phenotype while the beta produces biennis characters.
Biennis 3	From Virginia and North Carolina through western Pennsylvania and New York.	Both the alpha and beta complexes produce the biennis phenotype.
Parviflora	Same as Biennis 2.	Narrow, hairless leaves, bent stem tip, and subterminal sepal tips carried by the beta complex. Alpha complex of two types, one producing strigosa, the other biennis characters.

* The alpha and beta complexes of each group are also characterized by distinctive chromosomal end arrangements.

Among the biennis 1 races a few are completely heterogamous, i.e., in outcrosses transmit the alpha complex only through the egg and the beta only through the pollen. Other strains may transmit the alpha as well as the beta through the pollen, and only the alpha through the egg. In still others both complexes come through the egg, but only the beta through the pollen. Finally a race may be isogamous, i.e., transmit both complexes through the egg as well as through the pollen.

When certain biennis 1 races are crossed, hybrids are obtained which combine the alpha complex of each parent. If a number of different races are intercrossed a whole series of alpha-alpha combinations may be obtained. Each of these is self incompatible. Furthermore, any two alpha-alphas thus obtained which have no parental race or only one in common are usually cross compatible. These results led to the conclusion that each alpha biennis 1 complex carries an incompatibility allele (S_1). Thus, in a biennis 1 race, pollen bearing the alpha complex will not grow in the styles possessing the same allele (i.e. the same complex). In self-pollinations the incompatibility allele acts as pollen lethal for the alpha complex, preventing the formation of alpha-alpha homozygotes. Pollen bearing the beta complex lacks an incompatibility allele and functions normally; eggs carrying the alpha complex are fertilized to produce alpha beta offspring. If beta eggs are also formed, beta-beta homozygotes are theoretically possible. Since the latter

do not occur, their absence can be explained in two ways. Either the beta eggs may be eliminated through megaspore competition, or the beta-beta homozygotes may not develop because of zygotic lethals. Recessive lethal mutations which occur in a complex-heterozygote are not eliminated through segregation; therefore, both the alpha and beta complexes may accumulate lethals. Lethals in the beta complex prevent its occurrence in the homozygous condition while in the alpha complex such lethals reinforce the effect of the incompatibility allele. Whether a similar incompatibility mechanism exists in phylogenetic groups other than the biennis group 1 is the central problem under consideration.

MATERIALS AND METHODS

Races investigated for the presence of incompatibility alleles are listed in Table 2. These are complex-heterozygotes belonging to the various phylogenetic groups and were kindly provided by DR. RALPH E. CLELAND. A more detailed study of the biennis groups 2 and 3 with respect to incompatibility factors has been carried out by SCHULTZ (1959). The work reported here has dealt largely with strigosa, parviflora, certain biennis 2 races, and some biennis 1 collections not previously analyzed.

Self compatibility was tested both by seed set and pollen tube growth. Although most of the hybrids used were naturally self-pollinating, on occasion the anthers lacked pollen. In order to be certain that pollination occurred, two to four flowers were always hand pollinated before the tip was bagged. For determining pollen tube growth the flowers were first emasculated and bagged. When the stigmas were mature (usually 24 hours later), each flower was removed below the ovary and placed in a small test tube of water. Pollinations were then made and the flowers placed in an incubator at 28°C. In each test four styles were ordinarily used; one of these served as a control, being pollinated with pollen known to lack an incompatibility allele. Four hours were sufficient for the pollen tubes to approach or reach the base of the style in all control pollinations, if the stigma was mature at the time of pollination. The styles were then fixed in acetic alcohol and processed according to the schedule described previously (STEINER 1956).

Cross compatibility was tested only by determining pollen tube growth and not by seed set. The percentages of apparently viable and shriveled, empty pollen produced by certain alpha-alpha hybrids were determined. In addition, the type of starch grains found in the pollen of selected hybrids was ascertained.

RESULTS

Design of compatibility tests

Crosses between strigosa races: In the biennis group 1, incompatibility alleles were detected through the self incompatibility of the alpha-alpha hybrids obtained by intercrossing races of this group. Although CLELAND (1954) notes that among the strigosa races alpha complexes rarely come through the pollen (necessary if alpha-alpha hybrids are to be obtained by intercrossing races within the

TABLE 2

Races under investigation and their geographical origin

		biennis 1	
Beaufort	North Carolina	grandiflora (DEVRIES)*	Alabama
Bestwater I	Arkansas	Indianapolis	Indiana
Bestwater II	Arkansas	Iowa I	Iowa
Chicaginensis	Illinois	Mifflintown	Pennsylvania
Citronelle	Alabama	Poplar Bluff	Missouri
		biennis 2	
Ashland D	Wisconsin	Micaville	North Carolina
Buck Creek	North Carolina	Routhierville	Quebec
Elma I	New York	Shulliana	New Jersey
Elma V	New York	Tonawanda I	New York
Galeton	Pennsylvania	Tonawanda II	New York
Hollis	New Hampshire	York Beach	New York
Kouchi	New Brunswick		
LaSalle I	New York		
		biennis 3	
White Top	Virginia		
		strigosa	
Brookston	Colorado	Iowa VI	Iowa
Cockerelli	Colorado	Iowa XII	Iowa
Fargo	North Dakota	Monett	Missouri
Forsberg	Colorado	North Colorado Springs	Colorado
Granger	Washington	Palmer Lake	Colorado
Haskett	Manitoba	Sutherland	Nebraska
Heber	Utah	West Estes Park	Colorado
Iowa II	Iowa		
		parviflora	
angustissima	New York	Manistique	Michigan
muricata	Europe	Nobska	Massachusetts
Iron Mt.	Michigan	Tidestromii	Maryland
		European and unclassified	
Camas	Washington	suaveolens	Europe
Cantabrigiana	Europe	Wakefield	record of origin lost
Lamarckiana	Europe		

* Not a true grandiflora race.

group), six strigosa races, Brookston, Fargo, Granger, Heber, Iowa 2, and Palmer Lake, were crossed in all combinations. Out of the 30 different crosses, 23 grew to maturity in the field. The remainder either did not germinate or died in the seedling or rosette stage. Since information regarding the specific traits which the various alpha and beta complexes of these races carried was not available, and since the alpha and beta strigosa complexes are very similar in phenotypic effect, identification of alpha-alpha combinations by phenotype was not possible. Instead, each plant in a culture was bagged to test for self incompatibility. If self incompatible plants did occur, they could later be determined cytologically to confirm their complex constitution.

With the exception of two crosses, Granger × Fargo and Heber × Fargo, all

offspring were self-fertile and presumably were alpha·beta combinations. From the progeny of the latter cross ten plants were grown, six of which proved to be self incompatible. The Granger × Fargo cross gave two self incompatible plants among a progeny of 40. Attempts were made to obtain the cytological configurations of these self incompatible plants in order to confirm the hypothesis that they represented alpha·alphas. Unfortunately, because of the advanced state of the plants and the greater difficulty that strigosa combinations present in determining chromosome configurations, this confirmation was not obtained.

Hybrids combining the alpha complexes of races under study with alpha biennis 1 complexes: The main approach to testing for the presence of incompatibility alleles, however, was to cross as female parent the race in question to a biennis 1 alpha·alpha and to test the offspring for self compatibility. The progeny from such a cross should carry the alpha complex from the race being tested combined with a biennis 1 alpha complex bearing an S_I (i.e., self incompatible) allele (Figure 1). For convenience these offspring will be designated as *derived* alpha·alphas in order to distinguish them from the F_1 alpha·alphas obtained by crossing two races. If a derived alpha·alpha is self-fertile, the alpha complex from the race under investigation must carry an S_F allele (self-fertility allele). On the other hand, if the plant is self incompatible, two interpretations are possible. The self incompatibility of the derived alpha·alpha may result from the presence of an S_I allele contributed by the race being tested. A second explanation is that the alpha complex of the race in question carries a gametophytic lethal. The term "lethal" will be used here to designate a genetic factor which prevents the germination and development of the pollen regardless of the genetic constitution of the stigma upon which the pollen is placed. An incompatibility allele is a pollen lethal only in a much narrower sense, namely, when the stigma upon which the pollen is applied possesses the identical allele in its genotype.

To obtain a biennis 1 alpha·alpha, for example, alpha Paducah·alpha Hot Springs:

alpha Paducah(S_4)·beta Paducah(S_F) × alpha Hot Springs(S_1)·beta Hot Springs(S_F)

Among the offspring will occur:

alpha Paducah(S_4)·alpha Hot Springs(S_1)

To test a race, for example, Galeton, for the presence of self incompatibility:

alpha Galeton·beta Galeton × alpha Paducah(S_4)·alpha Hot Springs(S_1)

Offspring:

alpha Galeton·(alpha Paducah·alpha Hot Springs)(S_4)

alpha Galeton·(alpha Paducah·alpha Hot Springs)(S_1)

(These are derived alpha·alphas.)

If self compatible: alpha Galeton carries an S_F allele.

If self incompatible: alpha Galeton carries either an S_I allele or a pollen lethal or both.

FIGURE 1.—Examples of crosses used to obtain F_1 and derived alpha·alphas.

Evidence allowing a choice between the incompatibility and the lethal hypothesis can be obtained by testing the cross compatibility between a series of alpha-alphas which have been derived from different races and which have in common the same alpha biennis 1 complex, and thus, the same S_1 allele. The remaining alpha complex in each hybrid of such a series has been contributed by a different race. When these alpha-alphas are tested for compatibility with one another, three types of results are theoretically possible:

1. A particular alpha-alpha used as a pollen parent is cross compatible with any alpha-alpha possessing an alpha complex from a different race.

2. An alpha-alpha is cross compatible with some of the alpha-alphas in the series and cross incompatible with others.

3. An alpha-alpha is cross incompatible with all other alpha-alphas.

These results can be interpreted as follows:

1. The alpha complex of the race being tested carries an S_1 allele; this allele differs from the S_1 allele carried by all other alpha complexes under investigation.

2. The alpha complex of the race carries an S_1 allele which is identical to that found in the alpha complexes of some, but not all other races.

3. The alpha complex carries a lethal which prevents the growth of the pollen. In such a case an S_1 allele may also be present in the complex, but masked by the lethal. An alternative interpretation might be that all alpha complexes of the various races possess the identical S_1 allele; this is, however, merely another definition of a lethal and hardly conforms to the concept of a gametophytic incompatibility allele system.

In order to be certain of obtaining an S_1 allele from the male parent, biennis 1 alpha-alphas (S_1S_1) were originally used as pollen parents of the derived alpha-alpha hybrids. Biennis 1 races, i.e., alpha-betas ($S_1S_{1'}$), could have been used, but even those which regularly transmit the alpha complex through the pollen in many crosses, fail to do so in others; further, the percentage of alpha transmission varies widely so that large progenies must be grown in order to have a reasonable chance of obtaining offspring carrying the alpha. On the other hand, if an alpha-beta were used, all progeny receiving the alpha complex would carry the identical S_1 allele. Use of a biennis 1 alpha-alpha as the male parent, although insuring the transmission of an S_1 allele to each offspring, nevertheless, complicates the progeny analysis. A biennis 1 alpha-alpha as a pollen parent contributes either of two S_1 alleles to a particular offspring, i.e., the condition that each derived alpha-alpha in the series carry the identical biennis S_1 allele would not be satisfied. Each progeny would be composed of two compatibility classes. This would be the case if the alpha complex from the race under investigation carried either a lethal or an S_1 allele. Before crosses for distinguishing between the two hypotheses mentioned above could be made among the derived alpha-alphas, each plant would have to be identified with respect to the specific S_1 allele which it carried. Fortunately a means of circumventing this extensive compatibility testing has recently been found. The synthetic hybrid, alpha Hot Springs-beta Camp Peary L, was discovered to transmit almost always only the alpha Hot Springs complex

through the pollen except when alpha Hot Springs is present in the female parent and the incompatibility allele acts.

Thus, by crossing alpha Hot Springs·beta Camp Peary L as a pollen parent to the races under investigation, it became possible to obtain a series of alpha·alpha hybrids each of which carried an alpha complex from a different race in combination with alpha Hot Springs. The nearly complete transmission of alpha Hot Springs through the pollen made it possible to grow a relatively small number of offspring and still be certain of obtaining the desired combination. Because this method was first utilized only during the past growing season, the data obtained through its use are as yet limited.

Results of crosses of races with biennis 1 alpha·alphas

Crosses of this type are listed in Table 3. The data in this table have been partially summarized. From one to several different biennis 1 alpha·alphas were used as the male parent with each race; the number of different crosses are shown in the third column of the table. The biennis 1 alpha·alphas used in these crosses were:

alpha Birch Tree 1·alpha Walkerton
alpha Birch Tree 1·alpha Paducah
alpha Birch Tree 1·alpha Hot Springs
alpha Camp Peary E·alpha Walkerton
alpha Walkerton·alpha Birch Tree 2
(alpha Paducah·alpha Birch Tree 1)·(alpha Walkerton·alpha Birch Tree 2).

These biennis 1 alpha·alphas all exhibited seven pairs of chromosomes at meiosis; thus the two alpha complexes recombined freely, and each spore carried one or the other of the two different S_I alleles present in the plant. The progenies of the above crosses, therefore, were composed of two compatibility classes. Members of the same progeny also differed in phenotype since the pollen from which the progeny was derived consisted of recombinations of the two alpha biennis 1 complexes. With respect to chromosomal end arrangement, however, the genome contributed by biennis 1 alpha·alphas was identical in all crosses.

The majority of the derived alpha·alphas listed in Table 3 proved to be self incompatible. A few races, nevertheless, were shown to possess alpha complexes

TABLE 3

Crosses between races and biennis 1 alpha·alphas

Alpha biennis 1 with alpha complex of:	Self compatibility	Number of different crosses	Chromosome configuration
Angustissima	—	1	⊙ 10, 2 prs.
Ashland D	—	4	undetermined
Beaufort	+	2	⊙ 4, ⊙ 4, 3 prs.
Bestwater I	—	3	⊙ 6, 4 prs.
Bestwater II	—	2	⊙ 6, 4 prs.
Brookston	—	4	⊙ 10, 2 prs.
Buck Creek	—	4	⊙ 10, 2 prs.

TABLE 3—Continued

Crosses between races and biennis 1 alpha-alphas

Alpha biennis 1 with alpha complex of:	Self compatibility	Number of different crosses	Chromosome configuration
Camas	—	3	⊙ 8, ⊙ 4, 1 pr.
Cantabrigiana	—	2	⊙ 12, 1 pr.
Chicaginensis (excellens)	—	1	⊙ 4, 5 prs.
Citronelle	+	4	⊙ 4, 5 prs.
Cockerelli (curtans)	—	2	⊙ 8, ⊙ 4, 1 pr.
Elma I	—	5	⊙ 10, 2 prs.
Elma V	—	5	⊙ 10, 2 prs.
Fargo	—	2	⊙ 10, ⊙ 4
Forsberg	—	2	⊙ 8, ⊙ 4, 1 pr.
Galeton	—	3	undetermined
grandiflora (neoacuens)	+	1	⊙ 8, 3 prs.
Granger	—	3	⊙ 12, 1 pr.
Haskett	—	3	⊙ 6, ⊙ 6, 1 pr.
Heber	+, —*	8	⊙ 6, ⊙ 4, ⊙ 4
Hollis	—	2	⊙ 10, 2 prs.
Indianapolis	—	2	7 prs.
Iowa I	—	3	⊙ 8, 3 prs.
Iowa II	—	1	⊙ 8, ⊙ 4, 1 pr.
Iowa VI	—	1	⊙ 10, 2 prs.
Iowa XII	—	2	⊙ 8, ⊙ 4, 1 pr.
Iron Mountain	—	4	⊙ 6, ⊙ 4, ⊙ 4
Kouchi	—	4	⊙ 12, 1 pr.
Lamarckiana (gaudens)	+	2	⊙ 12, 1 pr.
Lamarckiana (velans)	+	2	⊙ 8, 3 prs.
LaSalle I	—	2	⊙ 10, 2 prs.
Micaville	—	5	⊙ 6, ⊙ 4, 2 prs.
Manistique	—	2	⊙ 14
Mifflintown	—	4	undetermined
Moncton	—	2	⊙ 10, 2 prs.
Monett	—	3	⊙ 10, 2 prs.
muricata (rigens)	—	1	⊙ 10, 2 prs.
Nobska	—	3	undetermined
North Colorado Springs	—	3	⊙ 8, ⊙ 4, 1 pr.
Palmer Lake	—	2	⊙ 8, ⊙ 4, 1 pr.
Poplar Bluff	—	4	7 prs.
Routhierville	—	2	⊙ 12, 1 pr.
Shulliana (jugens)	—	2	⊙ 8, 3 prs.
suaveolens (albicans)	—	2	⊙ 12, 1 pr.
Sutherland	—	2	⊙ 6, ⊙ 6, 1 pr.
Tidestromii	—	2	⊙ 14
Tonawanda I	—	2	⊙ 10, 2 prs.
Tonawanda II	—	2	⊙ 10, 2 prs.
Wakefield	—	4	⊙ 10, 2 prs.
West Estes Park	—	4	⊙ 4, ⊙ 4, 3 prs.
White Top	—*	4	⊙ 4, ⊙ 4, 3 prs.
York Beach	—	2	⊙ 10, 2 prs.

* See text.

in which an S_F allele is present. One other result is of interest: in certain crosses involving the race Heber the offspring were self incompatible, while in others in which a different alpha-alpha was used, they were self compatible. The individual hybrids and their reactions are listed below:

	Self compatibility reaction
alpha Heber-alpha Birch Tree 1	−
alpha Heber-alpha Birch Tree 2	−
alpha Heber-(alpha Hot Springs-alpha Birch Tree 1)	+
alpha Heber-(alpha Hot Springs-alpha Paducah)	+
alpha Heber-(alpha Paducah-alpha Hot Springs)	+
alpha Heber-(alpha Walkerton-alpha Camp Peary E)	−
alpha Heber-(alpha Walkerton-alpha Birch Tree 2)	−
alpha Heber-(alpha Paducah-alpha Birch Tree 1 × alpha Walkerton-alpha Birch Tree 2).	+

All other derived alpha-alphas having the same race as female parent were uniform in their compatibility behavior regardless of the particular biennis 1 alpha-alpha serving as male parent.

Compatibility tests between alpha-alphas possessing the alpha Hot Springs complex in common

As previously pointed out, these tests were designed to differentiate between the lethal and the incompatibility hypothesis each of which explains the failure of the derived alpha-alphas to produce seed upon self-pollination. The results are shown in Table 4. Since all of the hybrids carried alpha Hot Springs, the combinations are designated in the table only by the race from which the remaining alpha complex was derived.

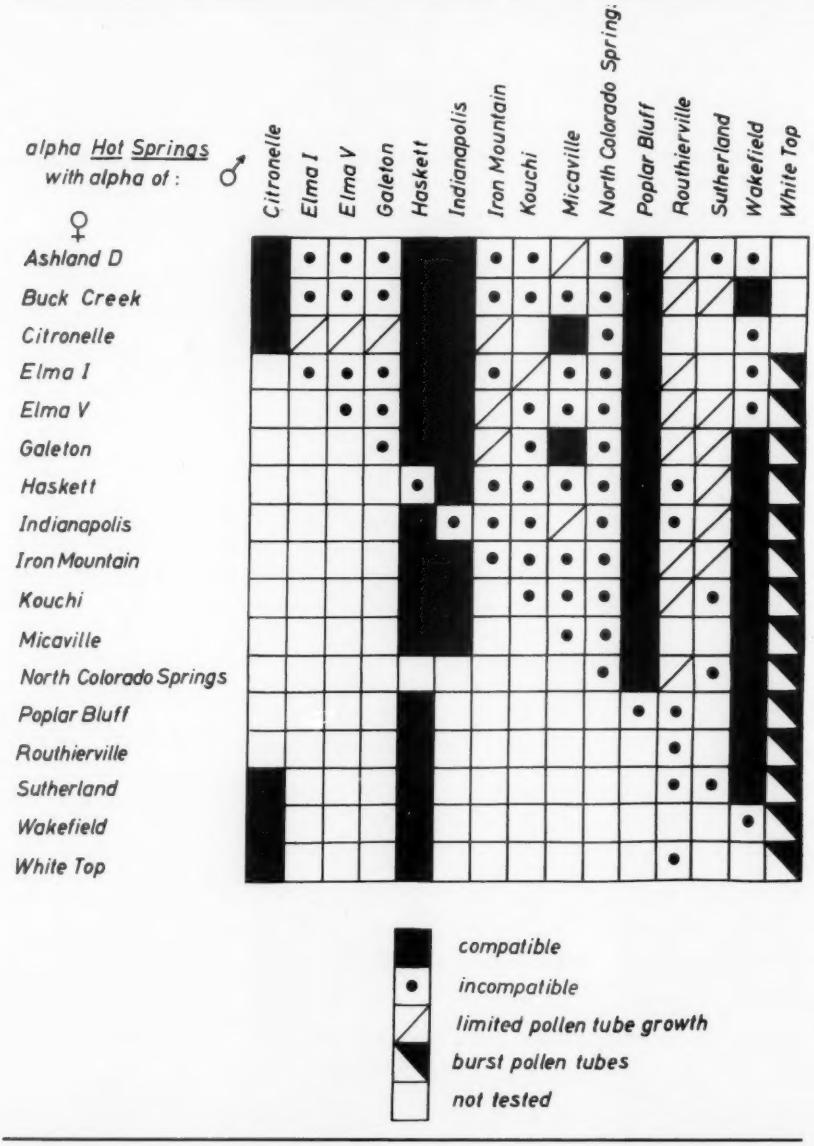
Citronelle, Indianapolis, and Poplar Bluff are biennis 1 races not tested previously. The behavior of Indianapolis and Poplar Bluff is consistent with that of other biennis 1 races; their alpha complexes are self incompatible but completely cross compatible with alpha complexes from all other races tested. Alpha-alphas carrying alpha Citronelle are self as well as cross compatible. Alpha Citronelle, therefore, must possess an S_F allele.

Alpha North Colorado Springs is consistent in its cross incompatibility when used as a pollen parent. The alpha complexes of Elma I, Elma V, Galeton, Iron Mountain, Kouchi, Routhierville, and Sutherland show limited pollen tube growth in at least one cross. In none of these cases, however, did more than a few tubes reach the base of the style. Alpha Micaville and alpha Wakefield were fully compatible in some crosses but not in others. Pollen carrying alpha White Top showed tube growth approximately one third of the distance down the style, but the tubes burst. Finally, alpha Haskett, from a strigosa race, was self incompatible but cross compatible in all combinations tested.

Those alpha-alphas whose pollen failed to develop in most or all of the crosses shown in Table 4 were also crossed to a series of races in order to test for com-

TABLE 4

Cross-compatibility between alpha alphas having the alpha Hot Springs in common



plete pollen sterility. With the exception of alpha Iron Mountain-alpha Hot Springs all consistently produced seed in the latter crosses. The functional pollen, therefore, must have carried the alpha Hot Springs complex. On the other hand, alpha Iron Mountain-alpha Hot Springs was outcrossed as pollen parent to 28 different races, but only five of these produced any seeds. With the races Friendship, Baltimore, Roanoke B, and Birch Tree 1 from six to 12 seeds were found in the capsules, while the cross to Chicaginensis produced approximately 50 seeds. Only in the cross with Indianapolis were fully filled capsules obtained. The latter cross was repeated; out of three pollinations two produced well-filled capsules. The pollen of alpha Iron Mountain-alpha Hot Springs consisted of 78.7 percent shriveled grains and 21.3 percent apparently good grains (2740 grains counted). The latter, however, contained spherical rather than spindle-shaped starch grains and fit the description of the "inactive pollen" described by RENNER (1919).

Inactive grains were not found among any of the other alpha-alphas shown in Table 4; these were the only hybrids whose pollen was examined in any detail. The percentage of apparently good pollen varied widely but tended to be higher in those alpha-alphas which were cross compatible with the others.

DISCUSSION

The majority of the alpha-alpha hybrids shown in Table 3 are self incompatible. These results led originally to the conclusion that the alpha complexes of phylogenetic groups other than the biennis 1 also carry incompatibility alleles (STEINER and SCHULTZ 1958). According to the incompatibility allele hypothesis, the alpha-alphas possessing alpha Hot Springs in common should be largely cross compatible provided that there are few or no S_f alleles in common. This is not the case, however; Table 4 reveals a considerable number of incompatible combinations. The failure of pollen carrying many of the alpha complexes under investigation to develop must result from the presence of pollen lethals. An alternative interpretation of the extensive cross incompatibility among other than alpha biennis 1 complexes is that all or most of the complexes carry identical S_f alleles. This seems unlikely for two reasons. First, it would be difficult to reconcile the widespread identity of S_f alleles in these forms with the fact that in the biennis group 1 almost every race possesses a different S_f allele. Further, as pointed out earlier, a situation in which identity of alleles was the rule would hardly meet the definition of a gametophytic incompatibility system. One is, therefore, inclined to favor the lethal hypothesis to explain the considerable cross incompatibility among the alpha complexes belonging to groups other than the biennis 1.

Nevertheless, this does not mean that incompatibility alleles are absent from these complexes. If a pollen lethal is linked to the incompatibility allele, the latter escapes detection. Unfortunately, most of the alpha-alphas under consideration form circles at meiosis and exhibit no more than three linkage groups, thus reducing the probability that the incompatibility factor and the pollen lethal will segregate.

Furthermore, there are several lines of evidence to support the conclusion that these complexes do carry incompatibility alleles which are masked by pollen lethals. In the first place, the majority of biennis 1 races do not transmit their alpha complexes through the pollen in outcrosses and thus must also possess pollen lethals. When such a complex, for example, alpha Poplar Bluff, is combined with alpha Hot Springs, the combination is self incompatible but cross compatible as the male parent with other alpha-alphas carrying alpha Hot Springs (Table 4). The reason is that at meiosis the chromosome configuration of this hybrid is seven pairs, and segregation of the pollen lethal and the incompatibility allele carried by alpha Poplar Bluff occurs. Thus, if it becomes possible to combine alpha complexes of the other groups with an alpha of the identical segmental arrangement, segregation of the *S* allele and the lethal may be realized and the *S* allele identified either as S_I or S_F.

Secondly, the crosses of Heber and Granger with Fargo suggest that alpha Fargo does occasionally come through the pollen in outcrosses. Because the alpha-alphas so produced are self incompatible, an incompatibility allele must be present in alpha Fargo.

Among the strigosa races tested there is one whose alpha complex clearly possesses an incompatibility allele. The combination alpha Haskett-alpha Hot Springs was self incompatible, but proved to be cross compatible with all other alpha-alphas tested.

The biennis group 2 race, Shulliana, transmits its alpha complex, jugens, through the pollen in outcrosses; therefore, it cannot have a pollen lethal. When jugens is combined with an alpha biennis 1 complex, the hybrid is self incompatible. Although this alpha-alpha has not been used in cross compatibility tests, the prediction that it will be compatible with most other alpha-alphas seems justified. The conclusion follows that jugens has an incompatibility allele.

Alpha Micaville, another biennis 2 complex, also appears to possess an incompatibility allele, which is however identical to that of several other races. More extensive testing of this complex is, however, desirable to strengthen the evidence for such a conclusion.

The presence of an incompatibility allele in the alpha complex of each of these races belonging to two different phylogenetic groups makes it likely that other races in these groups whose alpha complexes possess pollen lethals also carry incompatibility factors. The occurrence of incompatibility in a few isolated races would be difficult to explain other than as a relic trait which originally had been widespread. In the biennis group 1 incompatibility alleles occur in addition to pollen lethals; there is no obvious reason why they should persist only in this group of complex-heterozygotes and not in the biennis 2 and strigosa groups. This raises the interesting question as to why such alleles have persisted in races which are known to possess pollen lethals. It may well be that the incompatibility allele performs some essential function in the organism apart from the prevention of the occurrence of homozygous complex combinations.

As additional collections are analyzed, it may be possible to find strigosa, biennis 2, and parviflora races whose alpha complexes possess segmental arrange-

ments identical with or close to other alpha complexes known to carry an S_I allele. If alpha-alpha hybrids can be obtained in which complete segregation occurs, and if the lethal is not closely linked to the incompatibility allele, it may be possible to demonstrate the presence of such alleles in more of the races belonging to these groups.

The significance of the partial pollen tube growth in many of the alpha-alpha intercrosses is difficult to determine. It can be argued that these simply represent cases where the pollen lethal action is delayed. Since relatively few of these tubes reach the base of the style within a period that is more than sufficient for compatible tubes to travel the same distance, this conclusion seems at least tentatively justified.

Several other observations call for explanations. For example, the variation in self compatibility behavior of the different alpha Heber-alpha biennis 1 combinations is puzzling and requires further study.

The pollen sterility of the alpha Iron Mountain-alpha Hot Springs hybrid appears to be the same phenomenon described by STUBBE (1959) in his studies of genome-plastome interaction in Oenothera. When a biennis 1 or a strigosa type genome is combined with the cytoplasm of a parviflora race, the apparently good pollen is inactive. STUBBE believes that at the origin of the parviflora races the alpha parviflora complexes such as alpha Iron Mountain (phenotypically strigosa) became pollen inactive as a result of the genome-plastome interaction. Thus, the hybrid, alpha Iron Mountain-alpha Hot Springs, represents a biennis 1 and a strigosa genome in parviflora cytoplasm so that none of the pollen should be active.

More difficult to explain is the fact that the pollen of alpha Iron Mountain-alpha Hot Springs is sufficiently fertile when applied to the Indianapolis race to produce well-filled capsules, while most other crosses were either completely sterile or resulted in only a few seeds. The progeny from the Indianapolis cross will be grown during the coming season and may give a clue to this problem.

It is interesting to note that only a few alpha complexes carry an S_F allele; it is, of course, possible that some of the complexes possessing pollen lethals may turn out to have S_F rather than S_I alleles, although in light of the earlier discussion one would not expect an appreciable number of such cases to occur. Other than the complexes of *Oe. lamarckiana*, the alphas which exhibit self compatibility show a close relationship to the grandiflora phylogenetic group. The true grandiflora group is believed to be composed of self-fertile structural homozygotes (STEINER 1952); the alpha complexes of Beaufort, Citronelle, and the complex, neo-acuens (from the grandiflora collection which DEVRIES made in Alabama) all belong to races which probably represent hybrids between a true grandiflora and a complex-heterozygote very likely belonging to the biennis group 1. The failure of their alpha complexes to possess incompatibility alleles is consistent with the evidence concerning their phylogeny.

The culture of large numbers of self incompatible hybrids provides an opportunity to obtain information about the extent of hybridization which may occur in natural populations of complex-heterozygotes. Although the seed production of

a self incompatible alpha-alpha is less than that of the corresponding self-fertile alpha-beta combination, it is nevertheless appreciable. There is, therefore, no doubt that a great deal of pollen is carried from plant to plant. The amount of hybridization in nature among complex-heterozygotes will depend in large measure upon the number of flowers whose anthers lack pollen and whose stigmas have thus not been self-pollinated before the flowers open. In the experimental garden this occurs frequently, especially in certain strains, not without annoyance when pollen is desired for crosses. The question is whether an appreciable number of flowers whose anthers are without pollen also occurs in natural populations. A survey of a small biennis 1 population has shown that as many as 17 percent of the flowers opening on a particular date may lack pollen. The opportunity for hybridization thus must be considerable. If the population represents a single selfed line, such hybridization would have no significance, of course, but since many of our collections which were taken from the same field have yielded races differing both in phenotype and chromosomal end arrangement, such variability may actually be maintained through hybridization.

Also of interest is the fact that a self incompatible plant does not begin setting fruit as soon as it comes into flower even though it may be surrounded by plants with which it is compatible and which are flowering abundantly. The pollinating agents possibly may not begin visiting the plant until a certain level of flowering is attained.

There is a noticeable difference between an incompatible pollination and the lack of pollination. When pollination fails to occur, the ovary abscises shortly after the flower wilts. In a self incompatible pollination the ovary may persist for several weeks and often shows a distinct enlargement.

One other noteworthy observation was that at one edge of the season's planting were four rows of cultures all of which were not only self incompatible, but also incompatible with one another. None of these cultures showed an appreciable seed set, although seed could be obtained readily through artificial pollination with compatible pollen. Whether this was the result of the pattern of movement of the pollinating agents must for the present remain a speculation. It is hoped, however, that with a more detailed study of natural populations such observations can ultimately be integrated with the genetic and cytological facts to give an accurate picture of the population dynamics of this interesting group of plants.

SUMMARY

Fifty-one races of Oenothera belonging to five different phylogenetic groups were investigated for the presence of incompatibility alleles. Other than in the biennis group 1 incompatibility alleles could be demonstrated unequivocally in the alpha complexes of one biennis 2 and two strigosa races. Evidence is presented, however, which supports the conclusion that the alpha complexes of many of the races investigated carry incompatibility alleles which are masked by the presence of pollen lethals.

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MODIFICATION OF MUTATION FREQUENCY IN MICROORGANISMS. I. PREIRRADIATION AND POSTIRRADIATION TREATMENT[1]

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DEMEREC (1946) demonstrated that a finite time elapses between application of mutagen treatment and expression of the treatment-induced mutations in the phenotype. Two explanations advanced to explain this time delay in mutation expression hold (1) gene mutation is induced immediately upon application of the mutagen to that particular gene and time lag is necessary to allow for synthesis of an altered enzyme, and (2) induction of mutation is not instantaneous but certain cellular synthetic products and chemical events are necessary for fixation of the mutation, thus requiring finite time. It is evident that finite time is necessary in any case for the synthesis of an altered enzyme following a mutation, thus these hypotheses are not mutually exclusive. Until recently it has not been so clear that finite time is required for establishment of a mutation within the genome.

WITKIN (1956), HAAS and DOUDNEY (1957, 1959), DOUDNEY and HAAS (1958, 1959), and others have presented evidence which establishes the fact that mutations induced by ultraviolet are irreversibly fixed only after an appreciable time delay following treatment. It has been further shown that the mutation induction process is completed before the first postirradiation cell division and that the entire process of fixation of a mutation is dependent upon postirradiation protein synthesis. Critical chronological analyses of the synthetic events have recently been presented by HAAS and DOUDNEY (1959) indicating clearly that the establishment of a mutation requires postirradiation synthesis of DNA as the final step in the mutation process but the *expression* of the mutation is dependent upon protein synthesis subsequent to DNA synthesis.

In addition to the effects of postirradiation events on the fixation and expression of mutations, observations by WITKIN (1956), HAAS and DOUDNEY (1957, 1959), and in our own laboratory have shown that supplementation of the immediate preirradiation growth medium of various microorganisms with RNA purine and pyrimidine precursors leads to an increase in mutation frequency induced by ultraviolet irradiation. Consequently a hypothesis has been advanced that precursors of nucleic acids, altered *in vivo* by UV, become involved as chemical intermediates in the induction of mutation (DOUDNEY and HAAS 1958). Since this hypothesis is based on data derived from several strains of *E. coli*, it

[1] This manuscript reflects the views of the author and should not be considered official Air Force policy.

becomes of importance to learn whether these phenomena are generally observed in a variety of microorganism types, or if bacteria are unique in this respect. This paper reports evidence suggesting that the hypothesis holds for a number of diverse and unrelated species.

MATERIALS AND METHODS

An unidentified strain of Streptomyces sp. was isolated from a soil sample and determined to be antibiotically inactive *vs.* standard test strains of *Micrococcus pyogenes* var *aureus*. Examination of several thousand colonies plated on casein hydrolysate medium showed only two colonial color types. Accordingly, these two apparently stable colonial types were subjected to mutation induction and fixation experimentation as follows:

The minimal medium was Pridham's basal medium plus one percent glucose and 0.5 percent K_2HPO_4 (PBM) supplemented with yeast extract one percent w/v to supply RNA precursors in the preirradiation phases (PBYE) or with casein hydrolysate (1.5 percent w/v) in the postirradiation phase (PBCH). The radiation source was a model 30600 Hanovia mercury-vapor lamp giving an output of about 100 ergs/mm^2/sec at wave lengths below 280 millimicrons at a distance from the source of 30 cm. Spores were irradiated in 2.0 ml aliquots contained in 50 mm petri plates. The usual precautions to prevent photoreactivation were observed. Mycelium-free spore suspensions were prepared and 50 ml aliquots were suspended in PBYE for incubation at 25°C on a reciprocating shaker. After 90 minutes the spore suspensions were quickly chilled and held for one hour at about 3°C to achieve population synchrony. Spores were centrifuged cold (3°–4°C) and were resuspended in PBYE (25°C) for another hour of incubation on the shaker. The spores were washed in cold water (3°–4°C) and samples were withdrawn for control plating and for examination to determine count and clumping. Suspensions were then divided into 2.0 ml aliquots and were irradiated as outlined above for a period of time previously determined to give 90–95 percent kill. Following irradiation, the spores were diluted 1:10 in either 0.9 percent saline (controls) or PBM (controls) or PBCH. After 90 minutes of incubation at 25°C, suspensions were serially diluted and plated. Plates were incubated in the dark for 72 hours and were then examined for colony count. Additional incubation to 96 or 120 hours was given, and colonies were then scored for color type.

RESULTS

Distribution according to color type of 18,756 viable spores from two independent platings is shown in Figure 1. Isolate types 1 and 2 (wild types) occur in approximately equal number, but appearance of four color mutant types in one plating and five in the other is interpreted as favoring the hypothesis that mutation frequency may be altered through appropriate management of spores prior and subsequent to application of the mutagen.

Additional experiments were initiated to ascertain if characteristics other than

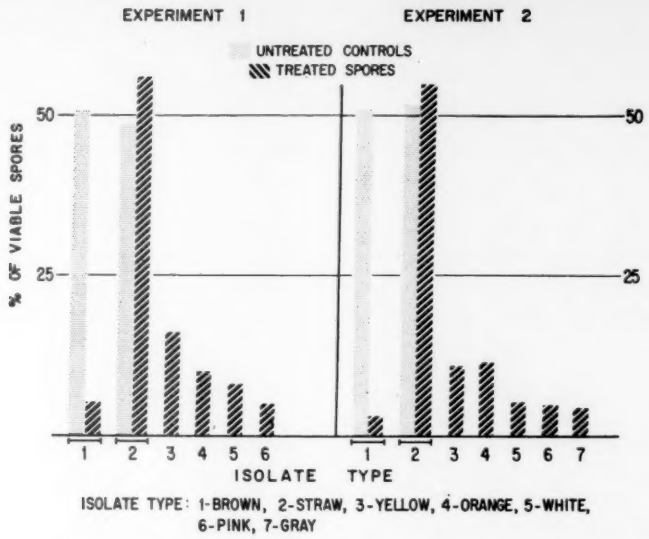

FIGURE 1.—Distribution of color mutants in Streptomyces sp., P-18. All isolates were grown from spores receiving 45 seconds of UV irradiation at the rate of 100 ergs/mm²/sec.

colony color might be concomitantly affected. The characteristic chosen for evaluation was antibiotic activity produced in fermentation broths shown by tests with *M. pyogenes* var *aureus*. In addition to 100 isolates from each group of wild-type colonies, 100 isolates from each of the mutant colony types produced in Experiment 2, Figure 1, were selected for antibiotic producing capability. Spore inocula from the isolates were placed in 50 ml glass vials containing 25 ml each of fermentation medium. Major components of the medium were milled oatmeal, beef extract, yeast extract, asparagine, sucrose, and KH_2PO_4. Broths were fermented five days on a reciprocating shaker, and aliquots of broth supernatant were tested for activity by the standard cylinder plate assay method. Results, summarized in Table 1, showed that none of the wild-type isolates from the control test produced any antibiotic activity at all while 3.56 percent of types 1 and 2 from the treated tests and 19.86 percent of the color mutants (types 3–7) showed antibiotic activity. These results are interpreted as further proof that the stated hypothesis is applicable.

A final test of the hypothesis in Streptomyces was designed to determine alteration of back-mutation frequency of a stable mutant. An arginine-dependent mutant of Streptomyces sp. (laboratory strain P-39) was selected. Its biochemical requirement was verified single and stable by repeated auxanographic analyses testing 40 pure substances including purines, pyrimidines, amino acids. RNA, DNA, and various carbohydrates. The minimal level of arginine required to permit very small but discrete colonies to form on PBM was determined. This level

TABLE 1

Premutagen and postmutagen treatment effects on antibiotic production

Spore group	Total no. tested	Isolate type	Antibiotically active* isolates recovered		
			Total no.	Percent of type	Percent of total
Control	100	1	0	0	0
	100	2	0	0	0
Treated	100	1	9	9.0	1.28
	100	2	16	16.0	2.28
	100	3	8	8.0	1.14
	100	4	8	8.0	1.14
	100	5	5	5.0	0.71
	100	6	44	44.0	6.29
	100	7	74	74.0	10.58

* A fermented broth was considered active if the zone of inhibition extended more than 1.0 mm past the edge of the test cylinder. For description of isolate type see Figure 1.

of arginine (0.002 percent w/v incorporated into all plating media) allows scoring of both prototrophs and auxotrophs on the same plates due to differences in colony size.

Ultraviolet killing curves, optimum preirradiation incubation, and optimum postirradiation incubation times were determined. Optimum preirradiation incubation time was determined to be two hours in PBCH plus minimal arginine supplement. Irradiation for 60 seconds gave 90–95 percent killing of spores. These conditions were imposed in all subsequent experimentation. Optimum postirradiation incubation is indicated on the curve of Figure 2 as 90 minutes.

Experimental spore populations were incubated two hours in PBYE before cold shock synchronization. Subsequent irradiations were carried out with the lamp output at 105 ergs/mm^2/sec at 30 cm from the source. After the prescribed postirradiation incubation, the spores were washed with distilled water and were plated on PBM plus minimal arginine. The usual precautions to prevent photoreactivation were observed. Control spores were treated in the same manner except that preirradiation and postirradiation incubation was done in PBM. Plates were incubated in the dark 96 hours prior to counting and scoring colony size. Results, summarized in Table 2, showed an average increase in frequency of back mutation to arginine independence of 309.9 times that of the controls. These data corroborate the qualitative findings regarding colony color and antibiotic production mutations and are interpreted as conclusive evidence in favor of the hypothesis under test.

DISCUSSION AND CONCLUSIONS

The broad interpretation of data presented is that, under conditions cited, actinomycetes conform in their genetic behavior to predictable patterns based on a hypothesis that nucleic acid precursors altered by ultraviolet action *in vivo* become involved as chemical intermediates in mutation induction. The data further support the opinion that the primary mutagenic action of ultraviole+

irradiation in actinomycetes is to alter purines and pyrimidines prior to their participation in biosynthesis of self-replicating material. A subsequent article will show that the altered precursors actually become incorporated into RNA and DNA moieties since UV altered constituents may be replaced by a chemical analog of uracil.

Comparisons of control data with results from treated spores (Tables 1 and 2) clearly show dependence of mutation rate alteration upon careful environmental control throughout the entire manipulative procedure. Furthermore, the essential manipulations must be accurately timed to achieve maximum mutation expression. Events under this protocol leading to mutagenesis are: (a) Saturation of the spores with RNA precursors during the preirradiation incubation. (b) Synchronization of many of the spores so that a large fraction of the population is susceptible to the mutagen at the time it is administered. Synchrony is probably the most critical condition to be achieved because preliminary data showed that the mutation rate varied directly with the degree of synchrony. (c) Controlled application of the mutagen to produce large concurrent killing and mutagenic effects. Quantitative analyses of the cause(s) of UV lethality have not been undertaken. (d) Postmutagen incubation in media containing luxury amounts of phosphorus and amino acids before plating allows time for RNA, DNA and protein synthesis. Stabilization and fixation of mutations are directly dependent upon this incubation period as shown by Figure 2. Maximum mutation response occurs only after about 90 minutes of postmutagen incubation. If the time is extended past 100 minutes a slow decline in mutation rate is exhibited. This observation suggests that, although the processes and syntheses required for induction of mutation occur prior to DNA synthesis, the final stabilization and fixation of the mutation in the genome are dependent upon DNA synthesis subsequent to irradiation.

Results summarized in Table 1 suggest, in addition to corroboration of the mutation hypothesis, a practically useful technique for alteration of antibiotic-producing capacity of actinomycetes. As the search for more highly specialized antibiotic agents becomes more difficult, the procedure outlined here should be a useful adjunct to methodology now in general use.

TABLE 2

Premutagen and postmutagen treatment effects on reversion to arginine independence

Spore group	Number of colonies scored	Reversions to arginine independence			
		Total no.	No./10^6 spores $\times 10^3$	Percent of group	Mutation increase*
Control	20,139	13	0.645	0.0645	....
Treated	22,389	4627	206.6	20.66	320.3
Control	25,755	18	0.698	0.0698	....
Treated	26,639	5650	212.0	21.20	303.7
Control	30,039	21	0.699	0.0699	....
Treated	29,909	6397	213.8	21.38	305.8
Totals	154,870	16,726		Avg.	309.9

* Percent treated/percent control.

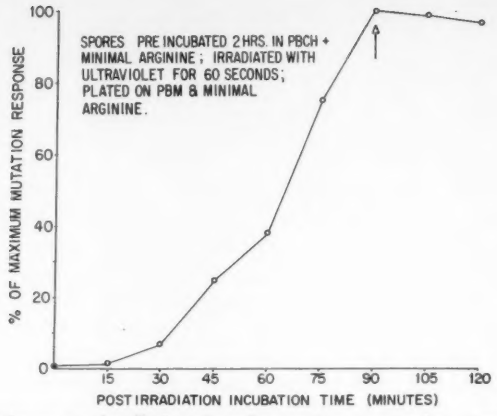

FIGURE 2.—Optimum postirradiation time for maximum mutation expression in Streptomyces sp.. P-39. The standard condition was selected as 90 minutes of postirradiation incubation.

SUMMARY

(1) Mutation frequency in Streptomyces may be altered through appropriate management of spores prior and subsequent to application of the mutagenic agent (UV irradiation). (2) The experiment protocol outlined produces similar alterations in frequency when the mutation criterion is colony color, antibiotic production, or reverse mutation from auxotrophy to prototrophy. (3) Data presented confirm the hypothesis that purine and pyrimidine precursors of RNA, altered *in vivo* by UV, become involved as chemical intermediates in the induction of mutations in Streptomyces sp. (4) The data further suggest that mutation induction takes place prior to DNA synthesis but that mutation stabilization, fixation and expression is dependent upon DNA and protein synthesis subsequent to UV irradiation. (5) A practically useful application of techniques described is indicated for utilization in alteration of antibiotic producing capacity of actinomycetes.

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THE RELATIONSHIPS OF THE CELLULAR ANTIGEN C OF COLUMBA GUINEA TO ANTIGENIC CHARACTERS IN OTHER SPECIES OF COLUMBIDAE[1]

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FOLLOWING the production of species hybrids between *Columba guinea* and *C. livia*, a segregation of cellular antigens which differentiate *guinea* from *livia* has been observed in the offspring resulting from backcrosses to *livia* of the species hybrids and selected backcross hybrids (IRWIN, COLE and GORDON 1936). Each of four such antigenic characters (A, B, C and E) has behaved in inheritance as a single character, in that the offspring from matings to *livia* of backcross birds carrying any one of them either have had the antigenic character *in toto*, or have lacked it entirely, in approximately equal proportions. This evidence indicates that each antigenic substance is the product either of a single gene or of linked genes between which crossing over has not yet been observed.

The transfer to *livia* of the respective chromosomes of *guinea* carrying genes which effect the antigenic characters which differentiate *guinea* from *livia* is only the first step in the study of the evolutionary patterns of these antigenic substances and their causative genes. Such patterns may be deduced in part from the appearance of identical or related substances in other species, as described elsewhere (IRWIN and MILLER 1961). More precise information has resulted from the tests for the relationships of one of the specific antigenic characters, C of *guinea*, among various species of Columbidae. The results of these tests are presented in this paper.

EXPERIMENTAL PROCEDURE

Antisera were produced in rabbits by injections of the cells of backcross birds heterozygous (C/C') for the respective causative genes for the C substance of *guinea* and C′ of *livia* (MILLER and BRYAN 1953) or derivatives of these backcross hybrids which are homozygotes (C/C). Six of 12 antisera against the cells of homozygotes (C/C) had antibodies for the C substance ranging in titer from 1:1920 to 1:15,360, and one of these (No. 594F1) was used in performing the

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majority of the tests to be described in this paper. Similar but not completely parallel results were obtained with reagents prepared from two other antisera (709F5 and 710F4) as described by BRYAN (1953). The absorptions of the antisera and the subsequent agglutination tests with the cells of the various species were carried out essentially as previously described (IRWIN and COLE 1936; IRWIN 1939).

The species which have been tested for their content of antigenic specificities related to the C of *guinea* belong to several different genera. Those of the genus Streptopelia are: *capicola, chinensis, humilis,* or *tranquebarica, orientalis, risoria, semitorquata, senegalensis,* and *turtur*. Those of the genus Columba are: *fasciata, guinea,* and *livia,* the domesticated form of the rock pigeon. Those of other genera are: *Chalcophaps indica, Ocyphaps lophotes, Phaps chalcoptera, Phaps elegans, Zenaida asiatica* (formerly called *Melopelia leucoptera*), *Zenaidura auriculata, Zen. graysoni,* and *Zen. macroura.*

Representatives of these species have been purchased from various dealers in the United States or have been obtained as described elsewhere (IRWIN 1953; IRWIN and MILLER 1961).

EXPERIMENTAL RESULTS

Comparisons of the erythrocytes from heterozygotes and homozygotes

Following matings *inter se* of backcross hybrids carrying the gene or genes for the C character, offspring were obtained which were homozygous for the causative genes. At first, the only criterion for the homozygosity of an individual was that all its offspring resulting from a mating to *livia* would contain the C character. Later, the use of the cells of the homozygotes (C/C) in absorption of anti-*livia* serum provided a reagent which was reactive primarily, and seemingly only, with the contrasting substance C′ in *livia* (MILLER and BRYAN 1953). Thus the respective reagents for C and C′ became available for use in differentiating the heterozygotes and homozygotes. A further test to differentiate the homozygote from the heterozygote depended upon the reactivity of cells of the heterozygotes (C/C') with a reagent for a hybrid substance (BRYAN and MILLER 1953). The cells of the homozygotes were not reactive with this reagent.

It will be noted from the data in the following insert that the interpretation appears valid that the C and C′ substances are respectively produced by single genes, or by two or more genes linked so closely that crossing over has not yet been observed. (For several years DR. W. J. MILLER was responsible for supervising these matings and for testing the offspring.)

	Offspring	
Kind of mating	With C	Lacking C
$C/C' \times C'/C'$	51	33
$C/C' \times C/C'$	49	13
$C/C \times C'/C'$	55	0
$C/C \times C/C'$	20	0
$C/C \times C/C$	12	0

All the birds were tested for the presence or absence of C, but only part of them were tested with the reagent for C′ of *livia*. The presence of C′ is therefore assumed, on the basis that C and C′ are antigens of a closed system (MILLER and BRYAN 1953).

Only rarely have definite differences been noted in the reactivities—agglutination or lysis—of the cells from heterozygotes and homozygotes. WIENER (1943) cites the experimental work which indicates that no differentiation between AA and AO, or BB and BO bloods has been possible, but that slight differences in reactivity have been noted between bloods from AB or MN individuals and those of their respective homozygotes. OLSON (1943) noted a quantitative difference in the reactivities of chicken cells from homozygotes and heterozygotes, and STORMONT (1952) found differences in reactivity and in absorbing capacity between the cells from homozygotes and heterozygotes in two genetic systems—FV and Z—of cellular antigens in cattle.

Definite differences in reactivity of the cells of the heterozygotes (*C/C′*) and those of the homozygotes (*C/C* and *C′/C′*) have not been observed in tests with the respective reagents from the different antisera (anti-*guinea*, anti-*livia*, anti-CC and anti-CC′). However, one of us (BRYAN) noted that a smaller quantity of cells from the homozygotes (*C/C*) than from the heterozygotes (*C/C′*) was required to exhaust an anti-CC serum of antibodies. An example of these tests with one (No. 710F4) of the three antisera to cells of homozygotes, with which such comparative tests were made, is given in Table 1.

It may be noted in Table 1 that a smaller quantity of cells of the homozygotes than of the heterozygotes was required to remove antibodies for the different test cells from the antiserum. Results varying only slightly from those of the table were obtained in parallel tests of the absorption of two other antisera against the cells of homozygotes (BRYAN 1953). One discrepancy in results is puzzling; there was a slight but definite reactivity of the cells of both homozygotes and heterozy-

TABLE 1

Differential absorbing capacities of cells of backcross birds homozygous or heterozygous for the gene(s) for the C character

Reactions of anti-CC serum (710F4) following absorptions with varying volumes of cells from birds homozygous (*C/C*) or heterozygous (*C/C′*) for the causative genes for the C substance

Time of absorption (minutes)	Volume of anti-CC serum (1:60)	Volume of cells in absorption	Cells tested and titers			
			livia (*C′/C′*)	*guinea* (*C/C*)	*C/C*	*C/C′*
Unabsorbed	....	....	9*	8	10	8
5	1 ml	1 ml	4/5†	3/4	5/8	4/7
5 + 30	1 ml	2 ml	0/0	0/1	1/6	1/3
5 + 30 + 30	1 ml	3 ml	0/0	0/0	0/4	0/2
5 + 30 + 30 + 30	1 ml	4 ml	0/0	0/0	0/3	0/1

* The digits refer to the titers of the doubled dilutions of the reagents, beginning at 1:60. Thus 1=60, 2=120 . . . 10=30,720.

† The numbers in the numerators refer to titers of the test cells following absorption with the cells of homozygotes (*C/C*), those in the denominators refer to the titers following absorptions by the cells of heterozygotes (*C/C′*). Equal volumes of cells and of antiserum (1 ml) were used at each absorption.

gotes following two absorptions of the antiserum with CC cells, but no reaction was noted with the cells of *guinea* (*C/C*). (Results quite contrary to these have been obtained occasionally in parallel tests with other antisera to C, in that the cells of *guinea* were agglutinated but those of the homozygotes or heterozygotes were not.) A satisfactory explanation for the failure of the cells of *guinea* to be reactive with these fluids is not apparent. However, the conclusion appears valid that the erythrocytes of the heterozygotes possess completely the C of *guinea* and the C′ of *livia* (MILLER and BRYAN 1953), but seemingly in lesser amounts than in the respective homozygotes. In addition to C and C′, the cells of the heterozygotes carry the "hybrid substance" (BRYAN and MILLER 1953).

The antigenic specificities, or antigenic groupings, of the C antigen of guinea

The agglutination reactions of the cells of the various species which were tested with the reagent for the C character of *guinea* prepared by absorption of an anti-C serum with the cells of *livia*, and with this reagent further absorbed with the cells of each of the species listed, are given in Table 2. The reagent prepared from antiserum 594F1 was reactive in varying degrees with cells of other species of Columbidae than are listed in the table, as *Caloenas nicobarica, Ducula spilorrhoa, Gallicolumba luzonica, Geopelia humeralis,* and *Goura cristata.* Unfortunately, the cells of these five species were not available for tests other than with the reagents for C of *guinea.* (We are indebted to DR. W. J. MILLER for making the cells of three of these five species available for these tests, and to MR. KARL PLATH, Curator of Birds, Chicago Zoological Park, Brookfield, Ill., and to MR. R. M. PERKINS, Director, Lincoln Park Zoological Gardens, Chicago, Ill., who kindly allowed blood to be taken by DR. MILLER from certain of these three species.)

Similar but not parallel reactivities to those given in Table 2 (line 1) were obtained in testing the cells of these 20-odd species with the reagents for the C character prepared from two other antisera, 709F5 and 710F4, following absorption with the cells of *livia.* The cells of *nicobarica, spilorrhoa, luzonica* and *humeralis* were not used in combination with these reagents. One of them, 709F5, was not reactive with the cells of *indica, cristata, chalcoptera, elegans* or *lophotes;* the other, 710F4, produced no observable reactions with the cells of *auriculata, chalcoptera, elegans* or *lophotes,* but both reagents were reactive with the cells of all the other species listed in Table 2.

There are a few reactions listed in Table 2 which are at variance either in different reactivities of the cells of a species with a given reagent at different times of testing, or in lack of agreement with the expectancy of the reactivities of reciprocal tests, as outlined by KRUMWIEDE, COOPER and PROVOST (1925). The interlocking relationships of the antigenic specificities (antigenic groupings) of the C substance in these various species are proposed despite these discrepancies.

The differential reactivities of the three reagents for the C character toward the cells of a few species suggested a complexity of the C substance, and a part of this complexity is revealed in the various reactivities summarized in Table 2. Thus, the cells of *indica* removed antibodies only for themselves from the re-

TABLE 2

The presence in various species of Columbidae of antigenic specificities related to the C character of Columba guinea

Reactions of the cells of the various species with the respective reagents prepared by absorption of antiserum 594F1 with the cells of *livia* either alone or in combination with the cells of the different species as listed:

Absorptions by the cell of:	*capicola*	*chinensis*	*humilis*	*orientalis*	*risoria*	*semitorquata*	*senegalensis*	*turtur*	*fasciata*	*guinea*	*C/C*	*livia*	*asiatica*	*auriculata*	*chalcoptera*	*elegans*	*graysoni*	*indica*	*lophotes*	*macroura*
livia	++	++	+	+	++	++	+	+	++	C	C	0	++	+	++	++	++	±	++	C
livia* + *capicola	0	+	0	0	±	+	?	0	+	C	C	0	++	0	+	+	+	0	++	++
livia* + *chinensis	+	0	±	±	±	±	++	0,+	+	C	C	0	++	±	++	++	++	0	++	++
livia* + *humilis	+	++	0	?,±	±	±	0,+	±	+	C	C	0	+	+	++	++	++	0	+	++
livia* + *orientalis	+	++	0	0	?,±	?,±	+	?	+	C	C	0	++	±	++	++	++	0	++	++
livia* + *risoria	+	++	±	±	0	0	0,±	?,±	+	C	C	0	+	0	+	+	+	0	±,+	+
livia* + *semitorquata	+	++	±	±	0,?	0	?,±	?,±	?,±	C	C	0	++	0	+	+	+	0	++	+
livia* + *senegalensis	+	++	±	±	±	±	0	±	++	C	C	0	+	±	++	+	+	0	+	+
livia* + *turtur	++	++	0	0	±	±	0	0	. .	C	C	0	++	0	+	+	+	0	++	++
livia* + *fasciata	+	++	0,?	0,?	0	0	0,±	0	0	C	C	0	++	+,0	+	+	+	0	+	+
livia* + *guinea	0	0	0	0	0	0	0	0	0	0	0,±	0	0	0	0	0	0	0	0	0
livia* + *asiatica	+	++	±	±	±,0	±,0	±	±	+	C	C	0	0	0	0	0	0	0	±	0
livia* + *auriculata	+	++	±	±	++	++	++	±	++	C	C	0	++	0	++	++	++	0	++	++
livia* + *chalcoptera	+	++	±	±	+	+	+	±	++	C	C	0	+	0	0	+	±	0	+	+
livia* + *elegans	+	++	+	±	+	+	++	±	+	C	C	0	+	0	0	0	±	0	. .	+
livia* + *graysoni	+	++	±	0,±	?,0	?,±	±	0	+	C	C	0	+	0,±	0,±	0	0	0	+	0
livia* + *indica	+	++	+	±	++	++	++	±	++	C	C	0	++	+	++	++	++	0	++	++
livia* + *lophotes	+	++	+	+	+	+	+	±	++	C	C	0	+	?,±	?	. .	±	±	0	±
livia* + *macroura	+	++	±	0,±	0,±	0,±	?,+	±	+	C	C	0	±	0	0	0	0	0	±	0
livia* + *orientalis* + *asiatica	±	+	0	0	0	0	+	±	++	C	C	0	0	0	0	0	0	0	+	0
livia* + *semitorquata* + *graysoni	±	+	±	±	0	0	±	±	+	C	C	0	±	0	0	0	0	0	±	0
livia* + *risoria* + *turtur	+	+	0	0	0	0	+	0	++	C	C	0	+	0	+	+	+	0	+	+
livia* + *fasciata* + *asiatica* + *chinensis* + *senegal* + *lophotes* + *capicola	0	0	0	0	0	0	0	0	0	C	C	0	0	0	0	0	0	0	0	0

Symbols: C=complete agglutination; ++=strong agglutination; +=agglutination; ±=weak but definite agglutination; ?=questionable agglutination; 0=no agglutination, at the first dilution of the reagent, usually 1:60. If two reactions are listed (as 0, ?; 0, ±; ?, ±), these were obtained at different tests.

agent for the C antigen, since the cells of all other species previously reactive with this reagent were still agglutinated following the absorption with the cells of *indica*. The antigenic specificity on the cells of *indica* related to the C of *guinea* is designated as "*a*." This specificity "*a*" is present on the cells of all the species listed in Table 2 except *lophotes*, because antibodies against the cells of *indica* were exhausted in each absorption except that by the cells of *lophotes*.

The cells of *humilis* exhausted the reagent of antibodies for themselves and for those of *indica*, and therefore contain at least one specificity "*b*" in addition to the *a* assigned to *indica; humilis* = *ab*. Similarly, the corpuscles of *orientalis* removed antibodies for the cells of both *indica* and *humilis*, as well as for themselves, so a third specificity "*c*" is present in *orientalis* in addition to the *a* and *b* of humilis; *orientalis* = *abc*.

The specificity *a* is present on the cells of *auriculata*, and also an additional specificity "*d*" which differentiated the reactivities of the cells of this species from those of *humilis* and *orientalis*. Similarly, *chinensis* carries *a* on its cells and a different specificity "*e*" from those previously assigned. The reactivities of *risoria* and *semitorquata* were parallel in these tests. Both species removed antibodies for the cells of each other and for those of *auriculata* (*ad*). The cells of both species were strongly agglutinated by the reagent made by the exhaustion of the antiserum with the cells of *auriculata*, so an additional specificity "*f*" is assigned to these two species, making them *adf*.

The cells of *turtur* exhausted the antiserum of antibodies for themselves, and also for the cells of *indica* (*a*), *orientalis* (*abc*) and *auriculata* (*ad*). Absorption by a combination of the cells of *orientalis* and *auriculata* (not given in Table 1) did not remove all the antibodies for the cells of *turtur*, hence an additional specificity "*g*" is assigned to *turtur* (*abcdg*). Further, the cells of *chalcoptera* and *elegans* removed antibodies for each other by absorption, and for those of *indica* (*a*) and *auriculata* (*ad*), but not for those of any other species. The antigenic specificity of the C character assigned to *chalcoptera* and *elegans*, in addition to *a* and *d*, is called "*h*". The cells of the two species, *graysoni* and *macroura*, were indistinguishable and presumably identical in their reactivities and capacity for absorption with this antiserum (with certain exceptions), exhausting it of antibodies for the cells of *indica* (*a*), *risoria* and *semitorquata* (*adf*) and probably of those for the cells of *auriculata* (*ad*), and *chalcoptera* and *elegans* (*adh*). Antibodies for the cells of *graysoni* and *macroura* were not removed by the pooled cells of *risoria* (*adf*) and *turtur* (*abcdg*), and the specificities *adfhi* are proposed as the minimum number in *graysoni* and *macroura* to explain the results.

The cells of *asiatica* exhausted the antiserum of antibodies for the cells of *graysoni* and *macroura*, among others, and were agglutinated by the reagents resulting from the use in absorption of the cells of either *graysoni* or *macroura* (*adfhi*), so an additional specificity "*j*", to those of the other two species, characterizes the cells of *asiatica* (*adfhij*). There are two species, *lophotes* and *senegalensis*, whose cells were agglutinated by each of the reagents prepared by absorption of the antiserum by the cells of each of the different species, with questionable exceptions. In absorption, the cells of *senegalensis* removed anti-

bodies for those of *indica*, whereas those of *lophotes* did not. A specificity in addition to the *a* of *indica* is assigned to *senegalensis* (*ak*), a different one to *lophotes* (*al*).

In view of the differing reactivities of the cells of *capicola* and *fasciata*, from each other and from all other species, and the agglutinations of the cells of the various species following the exhaustion of the antibodies from the antiserum by the cells of each of these two species, the specificities *abcdgm* are assigned to *capicola*, and *abcdfgn* to *fasciata*. Finally, since the cells of *guinea* in absorption removed antibodies from the antiserum for the cells of each of the species, but a combination of cells of the species (see last row of Table 2) carrying all the specificities *a* . . . *n* did not exhaust the antibodies for the cells of *guinea*, at least one additional specificity "*o*" is invoked for the cells of *guinea* or of backcross hybrids with the C character. Thus, 15 different specificities—*a b c d e f g h i j k l m n o*—are the minimum required to explain the relationships of the C character of *guinea* to related antigens on the cells of 17 species of Columbidae, as revealed by the interactions in absorption and agglutination with the antibodies in one antiserum to the C character.

BRYAN (1953) noted that two other antisera to the C character of *guinea* contained antibodies which by parallel analyses of the relationships of C-like substances in the various species revealed the same kind of pattern described above, but with respective differences obtained by the use of each antiserum. Presumably these differences in specificities of the antibodies and the specificities of the antigens with which they reacted are an index of the response of the individual rabbit to immunization with portions of a single suspension of blood cells. For example, the reagent for C prepared from one antiserum (No. 710F4) reacted with the cells of *indica*, that prepared from the other (709F5) did not. Further, the cells of *risoria* and *semitorquata* were indistinguishable in their reactivities with antibodies to C in antiserum No. 594F1, as described above, but were readily differentiated in tests with the other two antisera. The reactivities of the respective reagents prepared from the three antisera to C of *guinea* indicate strongly that the number of antigenic specificities proposed above for the C character is a minimum.

Unfortunately, other species of the genus Columba than are listed in the table were not available to be included in these tests. A previous report (IRWIN and MILLER 1961) has presented evidence that the antisera of seven of eight species of Columba, in addition to the three species included in the present report, contained antibodies to the C of *guinea*, and therefore these species undoubtedly share antigenic specificities of the C substance. Only one of these species, *palumbus*, appears to contain all or nearly all the antigenic specificities of the C character of *guinea*.

It seems reasonable to conclude that the antigenic substance or substances related to the C of *guinea* in the various species definitely share antigenic specificities with *guinea*, but that one or more other antigenic specificities may distinguish the C-like character in each species from the C of *guinea* and possibly from that of each other species. Examples of comparable relationships are those

of the d-1 substance of *chinensis* and the s-2 of *senegalensis*, which have been shown to share antigenic specificities as well as to possess those which differentiate each antigenic character from the other (IRWIN 1949; STIMPFLING and IRWIN 1960).

Further complexities of the C character of guinea

The C character has followed the pattern in the backcross populations which would be expected if it were effected either by a single gene or by linked genes between which a crossover has not yet been detected. No individual differences in respect to the C character have been noted among the backcross birds carrying the C of *guinea* since the antibodies for C were always completely removed from the antiserum by the calls of any such individual. This finding is not surprising because all backcross hybrids possessing the C character are the descendants of a single male of *guinea* (D172.1) which was mated to *livia* by the late PROFESSOR L. J. COLE before 1930.

However, observations over a period of years, and summarized in Table 3, have indicated definitely that the C character of *guinea* shares antigenic specificities with two genetically and serologically independent characters peculiar to *chinensis*, in contrast to *risoria*, *viz.*, the d-4 and d-11 antigens (IRWIN 1939), and to a character in *senegalensis* which is distinct from either of these two characters of *chinensis* (BRYAN 1953). Thus, the reagents respectively specific to d-4 and d-11 of *chinensis* were not reciprocally cross-reactive, but each agglutinated (Table 3) the cells of backcross hybrids with C of *guinea*, whether

TABLE 3

Tests of the antigenic relationships of the C of guinea *with the d-4 and d-11 of* chinensis *and the s-8 of* senegalensis

Anti-sera	Absorptions by the cells of	Test cells										
		chinensis	*guinea*	*livia*	*risoria*	*senegalensis*	d-4	E561G	d-11	*s*-8	CC′	CC
C of *guinea*	*livia*	+	++	0	+	0,+	+	..	+	0	++	++
C	*livia* and *risoria*	+	++	0	0	0,+	+	..	+	0	++	++
d-4	*risoria*	+	+	0	0	++	++	+	0	0	±	+
d-4	*risoria* and *guinea*	±	0	0	0	+	++	0,?,±	0	0	0	..
d-4	*risoria* and C/C′	±	0	0	0	++	++	?,±	0	0	0	0
d-4	*risoria* and E561D	..	0	0	0	±	±	0	..	..	0	..
d-4	*risoria* and d-4	0	0	..	0	0	0	0	..	..	..	..
d-4	*risoria* and *chinensis*	0	0	0	0	++	++	..	0	0	0	0
d-11	*risoria*	++	+	0	0	+	0	..	++	+	±	+
s-8	*risoria*	+	0	0	0	++	0	..	+	++	0	0

Symbols: Same as in Table 2.

homozygous or heterozygous. (Not all the reagents from antisera prepared against the cells containing d-4 or d-11 were reactive with cells carrying C, nor were all reagents from anti-C sera reactive with either the d-4 or d-11 antigens, rarely with both.) In view of the antigenic and presumed genetic relationship between d-11 of *chinensis* and s-8 of *senegalensis* (IRWIN 1949) it is somewhat surprising that cross-reactivity has not been observed between C of *guinea* and s-8 of *senegalensis* (Table 3). Seemingly, the antigenic grouping of d-11 which is shared with C of *guinea* is at least a part of the d-11 antigen which is not shared with s-8. Specificities *a* and *e* of the C antigen were assigned above to *chinensis*. If the specificity of the C of *guinea* called "*e*" is shared with d-11 of *chinensis*, an additional specificity is shared with *chinensis* by virtue of the relationship of C to the d-4 antigen of *chinensis*.

As is given in Table 3, the specific reagent for C was reactive with the cells of some individuals of *senegalensis*, but not with those of others. The cells of 11 birds of this species were reactive with this reagent, those of seven others were not. The presence or absence of this reactivity was independent of the presence or absence of the s-4 of *senegalensis* (previously called s-6, an homologous substance with d-4; IRWIN 1949). Of 11 birds whose cells were reactive with the reagent for C, seven carried s-4 and four did not; of the seven birds whose cells were not reactive with the C reagent, six carried s-4 and one did not (this reagent for C did not agglutinate cells with d-4 or s-4). Thus it appears that the C substance of *guinea* is cross-reactive with two of the independently occurring antigenic components of *chinensis* (d-4 and d-11) and also with two components of *senegalensis* (s-4, which is indistinguishable in backcross birds from d-4, and another component). BRYAN (1953) reported that the C of *guinea* was probably related to s-10 of *senegalensis*. No reagent specific only for s-10 has ever been obtained, so the identity of the antigenic character of *senegalensis* (not s-4) which is responsible for the reactions of some individuals of *senegalensis* with the reagent for C is not known.

The data of Table 3 show further that one backcross hybrid (E561G), the offspring of a mating to *risoria* of a backcross bird carrying only d-4 of *chinensis*, did not possess all the antigenic components of other backcross birds with d-4. That is, the cells of this bird did not by absorption remove all the antibodies from a potent d-4 antiserum for the cells of other birds (including a sib) carrying d-4. Presumably this bird resulted from either a crossover between linked genes effecting d-4, or a mutation. Two other examples of this kind have been noted in less than 250 backcross birds carrying this character, suggesting strongly that crossing over of linked genes is a more rational explanation of the fractionation of this substance than mutation. It may be noted in the data of Table 3 that the cells of this exceptional bird (E561G) removed all, or nearly all, the antibodies from a d-4 antiserum for the C character of *guinea*, whether in backcross birds or in *guinea*, but that a faint reactivity for the cells of E561G usually was observed after absorption of the antiserum by cells with the C antigen. Thus a major part, but not all, of the d-4 antigen of *chinensis* appears to be shared with C of *guinea*, and that part was carried on the cells of the exceptional individual, E561G.

DISCUSSION

The C antigenic character of *guinea* and the C′ of *livia* appear to be contrasting characters, according to the genetic behavior of each in the backcross population (IRWIN *et al.* 1936; MILLER and BRYAN 1953; and unpublished data). The progeny of *inter se* matings of backcross hybrids carrying the C antigen also conformed to genetic expectation on the assumption that C and C′ are single characters. However, the data presented in this paper show that the C antigen of *guinea* is antigenically related to each of two genetically and immunologically distinct antigens of *chinensis*, d-4 and d-11, and to an antigen of *senegalensis*, possibly s-10, (BRYAN 1953) also distinct from both d-4 and d-11.

If a single gene in *guinea* produces the C antigen, one would explain the cross-reactivities of C with the d-4, d-11 of *chinensis* and (possibly) s-10 of *senegalensis* on the assumption that these cross-reactivities were due to a fortuitous production of related antigenic groupings by the respective causative genes in each of the species. On this basis, one would expect that there should be cross-reactivity between each pair of the three antigens—d-4, d-11 and s-10—as well as between each of them and the C antigen. No cross-reactivity has been noted between the two antigens for which specific reagents are available, d-4 and d-11, according to the summary of these tests given in Table 3. If then, a single gene effects the C character, the cross-reactivities with the three different antigenic characters seemingly must be by virtue of the relationship of each of three different antigenic groupings of C to the respective differing antigens of the two other species (*chinensis* and *senegalensis*). It is possible that the relationship of C to both d-4 and d-11 suggests that d-4 and d-11 may be related, as proposed by MILLER (1956), but these findings may also be explained by assuming that genes at more than one locus in *guinea* are concerned in the production of the C antigen. This latter explanation appears more probable in the light of the available evidence.

If genes at two, possibly three or more, loci in *guinea*, with products related to the different antigens (d-4, d-11 and s-10) are acting together in *guinea* to effect the C antigen, one may question why crossing over has not been demonstrated in the backcross progeny. One possibility could be that the pertinent homologous chromosomes of the two species, *guinea* and *livia*, have accumulated inversions which effectively suppress crossing over between them. (The number and small size of the chromosomes in Columbidae have prevented extensive cytological analysis of the species and species hybrids.) Inversion differences may often exist between closely related species, but not always, according to DOBZHANSKY (1941, 1951), and PATTERSON and STONE (1952).

If the C of *guinea* is produced by a single gene, the relationships of the C-like substances in the other species of Columbidae to the C of *guinea* and to the C′ of *livia* could be satisfactorily explained by assuming that the causative genes in the various species constitute an allelic series. Of the species tested, only *palumbus* contains a substance indistinguishable from and presumably identical to C of *guinea*, implying then that the gene for C in *palumbus* is the same as in *guinea*

(IRWIN and MILLER 1961). In the other species, the presumed alleles would effect antigens related to C of *guinea*, as has been noted for a related group of antigens of another system in *chinensis*, *humilis*, *orientalis*, *senegalensis* and *guinea* (STIMPFLING and IRWIN 1960).

On the other hand, if genes at more than one locus in *guinea* effect the C character, it would seem reasonable to propose that at least two multiple allelic series would obtain among the various species to account for the observed relationships. The C of *guinea* is related to the independent characters d-4 and d-11 of *chinensis*, and to one in *senegalensis*, possibly s-10. Both d-4 and d-11 of *chinensis* are related to C of *guinea* and have been shown to be complex characters, by virtue of their relationships to related antigenic characters in various species of Columbidae (BRYAN 1953; PALM 1955). The most probable explanation of the interlocking relationships existing for the antigenic groupings of the C antigen to those of the antigenic characters in the other species appears to be to assume the existence in *guinea* and in the other species of two series of allelic genes. One series would produce antigenic groupings similar to the d-4 antigen, the other to the d-11 antigen. Other species of Columbidae might carry a gene in either or both series of allelic genes. This situation could, but need not, obtain also for the gene responsible for the antigenic grouping of C related to *senegalensis* (possibly s-10).

The experimental results show definitely that there are antigenic characters in species of Columbidae with varying degrees of relationship to the C of *guinea*. The assumption of the existence of two series of multiple alleles in these various species conforms to the genetic expectation that the members of an allelic series produce related effects, rather than essentially different effects of the final gene product.

At present very little information is available concerning the chemical bases of the specificities of the antigens of the red blood cells. The A and B substances of humans have been studied most intensively and these appear to be mucopolysaccharides—i.e., macromolecules containing carbohydrate chains and peptide units bound together (KABAT 1956). MORGAN and WATKINS (1959) indicate, as has been known for many years, that the specificities of the A and B soluble substances reside in the carbohydrate portion of the mucopolysaccharide molecules.

Extensive findings in the field of immunochemistry permit the general conclusion that antibodies to a particular antigenic substance may be, but need not be, reactive with a wide range of related chemical substances. Thus AVERY and GOEBEL (1929) noted that derivations of two simple monosaccharides—glucose and galactose—exhibited completely distinct immunological specificity when combined with proteins from widely separated species. In contrast, the antibodies against the α and β azophenol glucosides of glucose, when combined with distinct proteins, were cross-reactive (AVERY, GOEBEL and BABERS 1932). Many other examples of comparable cross-reactions of relatively simple but related chemical substances, made antigenic by attachment to proteins, are cited by LANDSTEINER (1945). OWEN (1959) has given examples of how the immunological reactions of

chemically related haptens (themselves not antigenic) attached to proteins may simulate the reactions of antisera to blood cells, prior to and following antibody absorption.

The possibility cannot be ignored that slight differences in the chemical makeup of relatively simple compounds may account for the antigenic differences which have been observed among related "antigens" of an antigenic system, such as the "B system" of cattle (STORMONT, OWEN and IRWIN 1951) or of the antigens in the various species of Columbidae which are related to the C antigen of *guinea*, or which belong in other antigenic systems. However, the authors believe that the chemical relationships among naturally occurring antigens may be more appropriately illustrated by invoking other examples. HARRIS and KNIGHT (1955) showed that there is a serological difference in both directions between normal tobacco mosaic virus (TMV) and TMV which had been treated with carboxypeptidase. The carboxypeptidase exclusively splits off threonine from the carboxyl ends of the peptide chains of the virus. Therefore, removal of the threonine groups (about 3000), comprising about 0.7 percent of the molecule, has changed the immunological specificity. Another example of a change in antigenic specificity of a protein molecule by virtue of a substitution, not a loss, of an amino acid has been reported by GOODMAN and CAMPBELL (1953) between normal adult hemoglobin and sickle-cell anemia hemoglobin. These examples suggest that definitive groupings play a major role in immunological specificity of proteins.

The cross-reactivities existing between certain types of the pneumococci may be fully as pertinent, possibly even more so, as examples of the possible chemical bases of cross-reactions of blood cells. Types III and VIII have long been known to be immunologically cross-reactive. The specific capsular substance of Type III is a polycellobiuronic acid, meaning that it is composed of glucose and glucuronic acid in definite linkage (GOEBEL 1935). The capsular substance of Type VIII has the same cellobiuronic acid units as part of its molecule, but it also has additional glucose and galactose. The reason for the reciprocal cross-reactivity of the antibodies to these two types is because of the occurrence of multiple cellobiuronic acid units in both capsular substances, although the linkages of the glucose and glucuronic acid units in the two capsular substances are different. According to HEIDELBERGER (1956) "there are many cross-reactions and it is possible now to show that these cross-reactions are due, in the case of the carbohydrate antigens, to similar groupings in similar linkages".

However, mere possession of the same basic units need not result in cross-reactivity, as exemplified by the presence of L-rhamose and D-glucose in both types II and XVIII, but no cross-reactivity has been noted between these two types (for references on this general topic see HEIDELBERGER 1956; see also for a table showing the content of polysaccharides in ten of the pneumococcal types). One may draw the general conclusion that immunological cross-reactivity of antigenic substances indicates a chemical similarity, but chemical similarity can exist without cross-reactivity.

Transformation of the pneumococcal types is recognized as having fundamental implications in biology, particularly in genetics, primarily following the

recognition of the role of desoxyribonucleic acid (DNA) in directing the transformation (AVERY, MACLEOD and MCCARTY 1944). The findings of EPHRUSSI-TAYLOR (1951) with variant forms of Type III indicate that six different transforming agents can be detected in the desoxyribonucleate of a single Type III smooth strain. Further studies of mutant types of both Types I and III by AUSTRIAN and BERNHEIMER (1959) and AUSTRIAN, BERNHEIMER, SMITH and MILLS (1959) have elucidated possible biochemical pathways concerned with the formation of uronic acids in the pneumococci. Analogous pathways to these may obtain in the formation of cellular antigens.

It is unfortunate that no information is available concerning the chemical nature of the cellular antigens in Columbidae. As stated above, the chemical basis of the cross-reactions among the pneumococcal types appears to the authors to be the most pertinent models of the possible chemical relationships of the cellular antigens, in the Columbidae and in other species. One can also visualize a possible explanation for the appearance of the hybrid substance, as in the heterozygotes (C/C'). As stated above, the same basic units of polysaccharides may be present in different types of the pneumococci, but without cross-reactivity being demonstrable. If one would then assume that in the respective homozygotes, C/C and C'/C', there exist one or more common antigenic groupings which do not engender specific antibodies, but in the heterozygote (C/C') these in some way became antigenically active, the appearance of a new antigenic specificity becomes understandable. However, a pertinent argument against this assumption is that an animal does not produce antibodies against the antigens of its own tissues. (The only known exceptions to this rule may occur because of isolation of the tissue from the antibody-forming cells.) Whether the new specificity is the result of an interaction between genes, possibly of alleles, or of an interaction between antigens, is still an open question.

SUMMARY

Backcross birds which carry only the C antigenic character of *guinea*, in contrast to *livia*, have been obtained by mating to *livia* the species hybrids and selected backcross hybrids from the mating of *guinea* × *livia*. *Inter se* matings of these backcross birds have produced offspring which are homozygous for the gene or genes producing the C antigen. The heterozygotes contain all of the C antigen of *guinea*, but less in amount than either *guinea* or C/C birds.

The reactions or lack of reactions of the cells of 18 species of Columbidae, following absorptions of antisera to the C antigen, showed clearly that the C of *guinea* is a complex character. The available evidence suggests that the C antigen of *guinea* is produced by the joint action of two, probably three, linked genes, based on the cross reactions existing between C and at least three genetically and serologically distinct antigenic characters in two other species.

Each of the species of Columbidae contained an antigenic character related to the C of *guinea* by one, usually two or more, antigenic groupings, or specificities, of the total of 15 antigenic groupings required as a minimum to explain the experimental results. The relationship of the causative genes in these species of

Columbidae producing the antigens related to C can be explained by assuming that the genes in the various species form a multiple series of alleles, belonging to one or the other, or both, of at least two such allelic series presumably existing in *guinea*, and that each member of the allelic series produces multiple antigenic specificities.

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PRODUCTION OF ALTERED Y CHROMOSOMES BEARING SPECIFIC SECTIONS OF THE X CHROMOSOME IN DROSOPHILA

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THE recovery and use of specifically marked Y chromosomes has greatly increased the versatility of the sex chromosome system of *Drosophila melanogaster* as a genetic tool. These Y's are marked by the normal alleles of a small heterologous segment of the genome. They have provided not only a simple means for following the Y chromosome through the meiotic divisions but also a useful tool for studying the segment of the genome for which the marked Y is duplicated. It is the second point that recommends the development of methods for accumulating a series of marked Y chromosomes.

For ease in referring to various marked Y chromosomes, we will establish the following conventions: (1) The long arm of the Y will be considered its left arm, so that when we write Y we infer $Y^L \cdot Y^S$, where the center point represents the centromere. (2) A marked Y will be symbolized by a combination of the symbol Y and the symbol of the marker commonly used; the marker symbol (e.g., m^+) will precede Y if it is located in Y^L, i.e., m^+Y, and follow Y if it is in Y^S, i.e., Ym^+. The symbol for the marked Y will not convey information about the origin or the relative positions of the genetic components of the chromosome. (3) The genetic constitution of a Y can be written *KL*·bb^+ *KS*, where *KL* and *KS* refer to the fertility complexes of Y^L and Y^S, respectively (BROSSEAU 1960). The constitution of a marker segment can be written a^+—b^+ where a^+ and b^+ are the extreme loci shown to be included in the segment. These two designations may be appropriately combined to describe a marked Y, e.g., *KL* a^+—b^+·bb^+ *KS* or *KL*(a^+—b^+)·bb^+ *KS* if the order of the a^+—b^+ segment is unknown.

Two marked Y's that have been widely available are the bw^+Y (DEMPSTER unpublished) and the y^+Y (=sc^s·Y of MULLER 1948). The bw^+Y is an X-ray-induced insertion of a subterminal section of 2R into the long arm of the Y chromosome probably proximal to the *KL* fertility complex. MULLER (1951) has shown that bw^+Y partially compensates for the Pale deficiency [*Df(2)P*] in that the combination +/*Df(2)P* does not survive whereas +/*Df(2)P*; bw^+Y does but produces a Minute phenotype. *Df(2)P* lacks a segment of 2R from the end of section 58E through 60D (BRIDGES 1937) or 60E1 (BRIDGES and BREHME 1944). GERSH (1956) has determined that the region of 2R inserted into the

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bw$^+$Y consists of at least the polytene bands 59E1·2 to 60E3. SLATIS (1955) has found that *bw* is situated between the bands 59D9 and 59E1·2; thus the region may be a few bands longer than the limits seen by GERSH. Since the right breaks in 2R for *bw*$^+$Y and *Df(2)P* practically coincide whereas the Pale deficiency extends much farther to the left than the *bw*$^+$Y duplication, the Minute phenotype characteristic of +/*Df(2)P; bw*$^+$Y is attributable to deficiency for a locus situated between the left breaks of these two rearrangements, i.e., *M(2)l*^{2}, (MULLER 1955). According to the cytological analysis of BRIDGES (1937), the region of 2R reported by GERSH to be inserted into *bw*$^+$Y carries, in addition to the brown locus, the loci of *mi*, *abb*, *pd*, *ll*, *mr*, *lx*, *l(2)NS*, *sp*, *bs*, *ba*, and possibly *M(2)33a*. We have tested *bw*$^+$Y in combination with *hv* (104.0) *bs*, *ba*, and *M(2)33a* and find that *bs* and *ba* are covered but *hv* and *M(2)33a* are not (see also MULLER 1955). Consequently, the segment of 2R inserted into Y^L extends from *bw*(104.5) to *ba*(107.4) inclusively, and the constitution of *bw*$^+$Y may be designated as *KL*(*bw*$^+$—*ba*$^+$)·*bb*$^+$*KS* (Table 1). The *bw*$^+$Y exhibits normal meiotic behavior and normal viability although two doses of *bw*$^+$Y are lethal.

The *y*$^+$Y (=*sc*^{8}·Y, MULLER 1948) arose as the consequence of an exchange between the distal inverted heterochromatic region of *In(1)sc*^{8} and the long arm of the Y chromosome distal to *KL*. Thus the distal uninverted euchromatic region of *In(1)sc*^{8}, bearing *l(1)J1*$^+$, *y*$^+$, and *ac*$^+$, was transposed to the terminus of Y^L. From detachment studies with the *y*$^+$Y, D. R. PARKER (personal communication) has determined that *bb*$^+$ from *In(1)sc*^{8} was not transposed to Y^L. An induced mutation from *y*$^+$ to *y* (=*y*^{53iY}) in the *y*$^+$Y was specifically sought and recovered by LÜNING (1953). Since this chromosome still covers deficiencies for *l(1)J1*, *y* and *ac* it is presumably *l(1)J1*$^+$ *y*^{53iY} *ac*$^+$ *KL*·*bb*$^+$ *KS* in constitution.

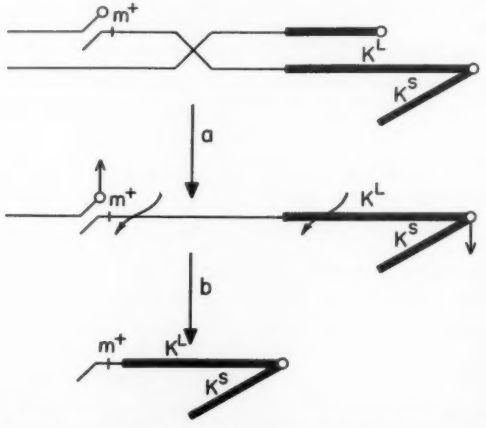

FIGURE 1.—The events required for the production of a Y chromosome marked with *m*+ from a T(1;4) broken just distal to *m*$^+$ and XY^L·Y^S. The straight arrows associated with the centromeres designate the distribution of elements at AI and the curved arrows the desired points of breakage for interstitial deletion.

TABLE 1

Tentative genetic constitutions of the existing marked Y chromosomes

Marked Y	Genetic constitution	Original designation and citation	
Singly marked			
4Y	*((ci⁺–spa⁺)KL)·bb⁺ KS*	Tp4;Y	MULLER and EDMONDSON 1957
B^SY	*B^S su^W-f⁺ KL·bb⁺ KS*	YB^S	BROSSEAU and LINDSLEY 1958
*bw⁺*Y	*KL(bw⁺–ba⁺)·bb⁺ KS*	Y:*bw⁺*	E. R. DEMPSTER, unpublished
*y⁺*Y	*l(1)J1⁺–ac⁺ KL·bb⁺ KS*	*sc^s*·Y	MULLER 1948
Y*ma-l⁺*	*KL·su^W-f⁺–sw⁺ bb⁺ KS*		
Y*w⁺*	*KL·bb⁺ KS spl⁺–kz⁺*		
Doubly marked			
B^S*w⁺*Y	*B^S su^W-f⁺ kz⁺—dm⁺ KL·bb⁺ KS*		
B^SY*y⁺*	*B^S su^W-f⁺ KL·bb⁺ KS ac⁺—l(1)J1⁺*		BROSSEAU 1958
bw⁺Yy⁺	*KL(bw⁺—ba⁺)·bb⁺ KS ac⁺—l(1)J1⁺*	*sc^s*·Y:*bw⁺*	COOPER 1952
y⁺YB^S	*l(1)J1⁺—ac⁺ KL·bb⁺ KS su^W-f⁺ B^S*		
y⁺Yw⁺	*l(1)J1⁺—ac⁺ KL·bb⁺ KS spl⁺—kz⁺*		
Triply marked			
y⁺Yw⁺B^S	*l(1)J1⁺—ac⁺ KL·bb⁺ KS spl⁺—kz⁺ su^W-f⁺ B^S*		

The marked ring Y of OSTER and IYENGAR (1955) is presumably of the same constitution as *bw⁺*Y. In general, the loci listed as being present have been accurately determined, but there may be additional loci that have not been detected, e.g., there may be *su^W-f⁺* as well as a mutant allele of *bb* between *ac⁺* and *KL* on *y⁺Yw⁺*. Furthermore, the order of elements may be in those derivatives that have an extensive history of irradiation.

The *y⁺*Y spontaneously mutated to produce the *y^{P59}*Y (MEYER 1959): the combination of *y^{P59}*Y with *y* in the X chromosome gives yellowish body and wings but dark bristles like *y*. The doubly marked recombinant *y⁺*Y/*bw⁺*Y (=*sc^s*·Y:*bw⁺*) was recovered by COOPER (1952) and subsequently shown by BAKER (1955, 1957), to be *KL(bw⁺—ba⁺)·bb⁺ KS ac⁺—l(1)J1⁺*. Apparently, the recombinant recovered by COOPER was between Y^L of the *y⁺*Y and Y^S of *bw⁺*Y. The *bw⁺Yy⁺* has been closed by OSTER and IYENGAR (1955) with the concommitant loss of *y⁺* to produce a ring Y still carrying the *bw⁺* segment.

A third marked Y, which to the best of our knowledge has not been widely used, is *Tp4*:Y described by MULLER and EDMONDSON (1957). This is an X-ray-induced transposition of all of chromosome 4 that is necessary for survival onto the Y chromosome. Two doses of this chromosome in the absence of any other Y- or 4-derived material produce viable and fertile individuals of both sexes. MULLER and EDMONDSON recovered a recombinant between this chromosome and Y^{S}X·Y^L that suggests that chromosome 4 is appended to or inserted into Y^L, and therefore in accordance with the conventions herein established this Y chromosome may be designated 4Y or *((ci⁺—spa⁺)KL)·bb⁺ KS* (STURTEVANT 1951; ABRAHAMSON, HERSKOWITZ and MULLER 1956, for chromosome 4 map data).

These marked Y's and their derivatives have been extremely useful, especially in studies of chromosome behavior and in radiation studies. This communication describes the production of several new marked Y chromosomes, one marked with *B^s*, one with *w⁺* and *N⁺*, and a third with *ma-l⁺*, by a method that theoretically may be used for production of Y's marked with any desired section of

the X chromosome. It must be emphasized that these marked Y's are derived by irradiation of complex rearrangements involving the X and the Y and cannot be considered to be normal Y's in any sense of the word. Their use must be tempered with the realization that the behavior of the Y itself is a variable that must be considered in any cross.

THE METHOD

This method requires the use of a reciprocal translocation that has a break in the X chromosome immediately distal to the region to be incorporated into the Y chromosome. One additional requirement of the translocation is that the second element must be broken sufficiently close to its tip that hyperploidy for this distal region will not adversely affect viability. Translocations with this second requirement represent an extremely restricted sample of T(X;2)'s and T(X;3)'s; furthermore, the male sterility that is commonly associated with T(X;2)'s and T(X;3)'s (SCHULTZ 1947; LINDSLEY, EDINGTON and VON HALLE 1960) drastically restricts their usefulness in the present scheme. For these reasons the method is nearly restricted to T(X;4)'s and T(X;Y)'s. It may seem that the restrictions placed on the chromosomal raw materials required for the production of marked Y's are so severe as to seriously limit the versatility of the method. However, a series of 86 T(X;Y)'s with X chromosome break points in every division of the polytene map has been produced and provides material for the production of a vast array of marked Y chromosomes (NICOLETTI and LINDSLEY 1960). We will describe the method, using as an example a T(X;4). The first step involves making the translocation heterozygous to $XY^{L.}Y^{S}$ (or $XY^{S.}Y^{L}$); these XY's were derived from detachment experiments by PARKER and MCCRONE (1958). An exchange proximal to the break point of the translocation replaces its proximal X heterochromatin with that of the XY (Figure 1a). The constitution of such recombinants may be designated as $X^{D}4^{P}$; $4^{D}X^{P}Y^{L.}Y^{S} = T(XY^{L.}Y^{S};4)$ (D = distal; P = proximal) and they may easily be balanced with attached-X's. Extra Y's are eliminated from the T(XY;4) stock by crossing males to XX/0 females. Consider for example a $T(XY^{L.}Y^{S};4)$ with the normal allele of a marker, m^{+}, just proximal to the break point in X. T(XY;4)/0 males produce four sperm types (1) $X^{D}4^{P}$; $4^{D}X^{P}Y^{L.}Y^{S}$, m^{+}; (2) 0 (3) $X^{D}4^{P}$ (4) $4^{D}X^{P}Y^{L.}Y^{S}$, m^{+}. Fertilization of X-bearing ova from *m* females will produce (1) X, $m/T(XY;4)$, $m^{+} = m^{+}$ daughters (2) X, $m/0$ = sterile *m* sons (3) X, $m/X^{D}4^{P} = m$ aneuploid (4) X, $m/4^{D}X^{P}Y^{L.}Y^{S}$, $m^{+} = m^{+}$ aneuploid; owing to lethality or sterility of the aneuploid classes, no fertile sons will result. The irradiation induced deletion majority of X^{P} from $4^{D}X^{P}Y^{L.}Y^{S}$ gametes can produce a derivative that approaches $4^{D}Y^{L.}Y^{S}$ in constitution, thus reducing the aneuploidy and consequently conferring viability or fertility or both upon $X/4^{D}Y^{L.}Y^{S}$ zygotes. The object of the present scheme is to recover such a deleted derivative that retains a distal remnant of X^{P} including m^{+} (Figure 1b). This product will be recognized as a fertile m^{+} son. With the exception of very rare patroclinous sons resulting from maternal primary nondisjunction, the only fertile sons of the above cross will carry a deleted $4^{D}X^{P}Y^{L.}Y^{S}$; if the parental flies are carefully discarded, their progeny may

simply be transferred to fresh medium and the production of larvae used to screen for those containing a fertile male. In a translocation where the X/$4^{D}X^{P}Y^{L}{\cdot}Y^{S}$ aneuploid is inviable, a fertile male with m^{+} may be recognized immediately in a fertile progeny. Where such aneuploids survive it is necessary to utilize the progeny of males carrying the deleted $4^{D}X^{P}Y^{L}{\cdot}Y^{S}$ to isolate this product.

The Bar Stone Y: We used T(1;4)B^{S} to construct the B^{S}Y chromosome according to the scheme described in the preceding section; B^{S}Y has B^{S} appended to the terminus of Y^{L}. The marked Y's available before construction of B^{S}Y are marked by normal alleles from some heterologous segment of the genome (except for the Hairy-wing effect of y^{+}Y), and their usefulness depends on rendering the remainder of the system homozygous for some recessive gene in the region homologous to the marker segment of the Y. Since this is often inconvenient, especially with bw^{+}Y, we decided to produce a Y marked with Bar (BROSSEAU and LINDSLEY 1958). B^{S}Y can be readily scored in all combinations except those in which the X carries *B*.

T(1;4)B^{S}, B^{S} *car*/$XY^{L}{\cdot}Y^{S}$, y^{2} *su-w*^{a} *w*^{a} *KL·KS* females were crossed to $Y^{S}X{\cdot}Y^{L}$, *y cv v*/0 males; B^{S} *car*$^{+}$ sons were selected. They could have resulted from exchange between *w*^{a} and the translocation break point with primary nondisjunction of the XY from the proximal piece of T(1;4)B^{S}, i.e., $XY^{L}{\cdot}Y^{S}/4^{D}X^{P}$, B^{S} *car*, in which case they would have been sterile owing to hyperploidy for the proximal portion of the X. Alternatively, they could have been recombinants between B^{S} and *car*, i.e., T($XY^{L}{\cdot}Y^{S}$;4)B^{S}; in which case, they would have been of the desired genotype and fertile. All recovered B^{S} *car*$^{+}$ sons were crossed to attached-X, *y v bb*/0 females, and several of them proved to be fertile. The fertile crosses produced balanced stocks consisting of *y v bb* and *y v* B^{S} daughters and B^{S} sons. Males from such a stock were irradiated with 4000r and crossed to *y v* females. Before the F_1 began to emerge the parental flies were discarded, and the F_1 was subsequently transferred into fresh culture bottles which were later examined for evidence of fertile males. As described in the preceding section fertile males in the F_1 are either T(XY;4)B^{S}/0 resulting from maternal nondisjunction or *y v*/$4^{D}Y^{L}{\cdot}Y^{S}$, which are *y v B* males and result from deletion of a portion of X^{P} from $4^{D}X^{P}Y^{L}{\cdot}Y^{S}$. From this experiment one such deleted $4^{D}X^{P}Y^{L}{\cdot}Y^{S}$ chromosome was recovered (Figure 2a) that carries *KL* and *KS*, a remnant of X^{P} carrying B^{S} and *su*^{w}-*f*$^{+}$ (ZIMMERING 1959) but not *ma-l*$^{+}$ or *sw*$^{+}$, and a portion of the tip of chromosome 4 not including the locus of *spa*, which ABRAHAMSON *et al.* showed to be the distal-most marker on chromosome 4. According to LEWIS (1956), the fourth chromosome break in T(1;4)B^{S} is in 102F. In T(X;Y)'s in which B^{S}Y has been broken in Y^{L} (NICOLETTI and LINDSLEY 1960) the tip of the long arm of B^{S}Y can be seen free of the chromocenter. It consists of three double bands; the distal-most has been seen to pair with the Bar region of the X and is probably 16A1; the proximal-most has been seen to pair with a band in region 20 (20A1-2 or 3) and is probably the *su*^{w}-*f* band. No bands of chromosome 4 are discernible. B^{S}Y segregates regularly from a free X or attached-X's and is characterized by good viability in X/B^{S}Y or X/B^{S}Y/B^{S}Y males and in X/X/B^{S}Y females.

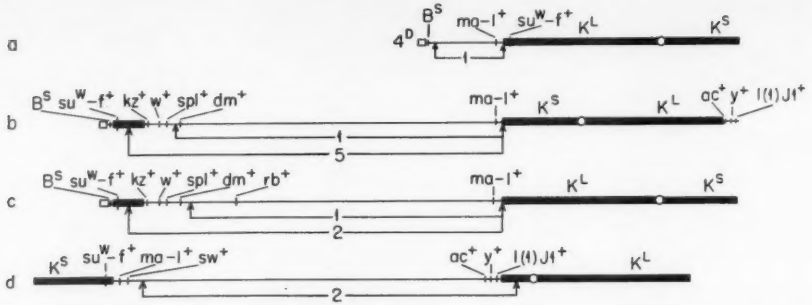

FIGURE 2.—The chromosomes irradiated to produce the marked Y chromosome recovered in the current experiments and the events proposed to account for their origin. The region bracketed between two arrows represents a deleted segment and the associated number indicates the number of such derivatives recovered. In each case only the half of the translocation in the irradiated sperm is figured. The thin line = X euchromatin; the open heavy line = autosomal euchromatin; the solid heavy line = Y or X heterochromatin.

BROSSEAU (1958) has recovered a recombinant between B^{S}Y and bw^{+}Yy^{+} that is B^{S}Yy^{+} (Table 1), and we have recovered a B^{S}Yy^{+11d} chromosome from an irradiated female that carried both the B^{S}Yy^{+} and a derivative of *In(1)sc*^{8} marked with y^{+11d}. These chromosomes have great potential utility for studies of detachment and of translocation or recombination involving the Y.

The white^{+} *Y:* A translocation between the X chromosome and the B^{S}Y [T(X;Y)148 (NICOLETTI and LINDSLEY 1960)] was used in the construction of the w^{+}Y. T(X;Y)148 is a reciprocal translocation with the breaks in region 2D of the X and in Y^{L} proximal to B^{S} but distal to all members of the *KL* fertility complex. This was shown by demonstrating that males containing Y^{D}X^{P} from T(X;Y)148 and X^{D}4^{P} from T(1;4)w^{m5} are sterile in combination with Y chromosomes that are separately deficient for *kl-1*, *kl-2*, *kl-3*, *kl-4*, and *kl-5*, the five components of the *KL* fertility complex demonstrated by BROSSEAU (1960). The T(XY;Y) recombinants were recovered from T(X;Y)148, *y* B^{S}/XY^{S}·Y^{L} (129–16), y^{2} *su-w*^{a} w^{a} *KS·KL* y^{+} females crossed to Y^{S}X·Y^{L}, *Ins(1)EN*,dl-49, *y v f car*/0 males. The desired recombinants were recognizable as y^{+} B^{S} males, and they were crossed to attached X y^{2} *su-w*^{a} w^{a} *bb*/0 females; their progeny consisted exclusively of y^{2} w^{a} females. In the absence of a free Y chromosome the two components of the translocation separate from each other with complete regularity; the y^{2} w^{a} daughters were hyperploid for X^{D}Y^{P}, which carries the normal allele of *su-w*^{a}, and when backcrossed to their fathers they produced a balanced stock in which the males are X^{D}Y^{P}; Y^{D}X^{P}Y^{S}·Y^{L}/0 and the females are XX/X^{D}Y^{P}. Males from this stock were irradiated with 4000r and crossed to *y w*^{a} *spl rb* females. Owing to the regular segregation of the two halves of the translocation, the zygotes produced are *y w*^{a} *spl rb*/X^{D}Y^{P}, *y KL·KS* which survive and are *y w*^{a} *spl rb* males with hyperploid characteristics (including sterility), and *y w*^{a} *spl rb*/Y^{D}X^{P}Y^{S}·Y^{L}, B^{S} *KS·KL* y^{+} which do not survive. Deletion of the ma-

jority of X^p leads to the production of sons that are y^+ and *B* and that may be w^+, depending on the break points (Figure 2b). A similar series of crosses were carried out with $XY^{L}\cdot Y^{S}$ (108–9), *y² su-w^a KL·KS* in place of $XY^{S}\cdot Y^{L}$ (129–16). Among ~ 12,000 progeny of irradiated sperm of the genotypes shown in Figures 2b and 2c, 11 fertile derivatives of $Y^{D}X^{P}Y^{L}\cdot Y^{S}$ and $Y^{D}X^{P}Y^{S}\cdot Y^{L}$ were recovered. One carried no markers other than fertility, one carried y^+ and fertility, and the remaining nine (which are explainable as interstitial deletions) are indicated in Figure 2b and c. The two derivatives marked with the normal allele of white, i.e., $y^+Yw^+B^S$ and B^Sw^+Y (Table 1), are characterized by normal meiotic behavior and normal viability. They show a dominant Confluens effect, and should be extremely useful in covering the lethal effect of Notch.

The triply marked $y^+Yw^+B^S$ has been irradiated, and a large number of fertile singly and doubly marked derivatives have been recovered. Among these are several y^+Yw^+ chromosomes. The y^+Y^L arm of one of these y^+Yw^+ chromosomes has been replaced with an unmarked Y^L by exchange in an $XX\cdot Y^L/y^+Yw^+$ female, where $XX\cdot Y^L$ is a reversed acrocentric compound X chromosome that carries an unmarked Y^L as a second arm. This recombinant, then, is Yw^+. Owing to the complex origin (through repeated irradiations) of these derivatives of the triply marked Y, their constitution as given in Table 1 is necessarily tentative. The order of elements is one that has been inferred from the events that are considered likely to have taken place.

*The Y*ma-l⁺: This Y chromosome was recovered by a different method from the one described earlier in this communication. $Y^SX\cdot Y^L$, *In(1)EN, KS B y·KL/0* males were irradiated and crossed to *ma-l* females. Two *ma-l*⁺ sons were recovered and found to be fertile. Tests showed that the Y chromosomes carried by these sons contained normal alleles of *su^w-f*, *ma-l*, and *sw*, but not of *car* or *l(1)J1*. This Y presumably arose through deletion of a segment of the XY from the right of *sw*⁺ (the left of *sw*⁺ in a normal sequence) to the right of *l(1)J1*; its constitution is *KL·sw⁺—su^w–f⁺ bb⁺ KS* (Figure 2d).

SUMMARY

A method for producing marked Y chromosomes by irradiating reciprocal translocations between an XY chromosome and some other element in the genome has been used and a series of marked Y's recovered. The most useful markers that have been appended to a Y are B^S, w^+ and N^+, and *ma-l*⁺. In addition to these Y's, which carry single marker segments, a number of doubly marked Y's have been constructed. Table 1 lists the current best estimate of the constitutions of the known marked Y chromosomes.

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VARIABILITY OF INCIDENCE AND CLINICAL MANIFESTATION OF MOUSE HEREDITARY MUSCULAR DYSTROPHY ON HETEROGENEOUS GENETIC BACKGROUNDS[1]

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AN hereditary muscular dystrophy (symbol *dy*) in the mouse (MICHELSON, RUSSELL and HARMAN 1955) appeared as a deviant in the strain 129/Re, which is highly inbred. All available evidence on the pattern of incidence of this abnormality in 129/Re pedigrees, including the descendants of 129/Re-*dydy* ovaries transplanted to normal host females (STEVENS, RUSSELL and SOUTHARD 1957), supports the hypothesis of an autosomal recessive factor. These data depend, however, upon the expression of the entity against the uniform 129/Re genetic background. No evidence has been published as yet demonstrating expression of the mouse dystrophy syndrome against varying genetic backgrounds. The hereditary muscular dystrophies of man are probably likewise caused by single factor mutations (WALTON 1955), but they occur on backgrounds of extreme genetic variation.

An investigation of the penetrance and expression of mouse muscular dystrophy on various heterogeneous genetic backgrounds was therefore undertaken. The investigation served a dual purpose: (1) to explore the range of variability of the mouse dystrophy syndrome, especially to determine if it would break up into various clinical entities under various conditions of genetic background; (2) to corroborate previous reports that mouse muscular dystrophy is caused by a single recessive mutation. Moreover, the segregating F_2 populations that gave rise to hybrid dystrophics provided the data for linkage tests.

TERMINOLOGY AND METHODS

In the present communication, the term "hybrid" is used with reference to heterogeneous F_2 animals resulting from the cross of two different parent strains or stocks. In a few instances, a more specific use is indicated by qualifying "uniform F_1 hybrid."

We propose to substitute the term *congenic* for *coisogenic* when referring to stocks produced by the introduction of a mutant into an inbred background by

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repeated backcrosses. In this connection the latter term has been used with qualifications by various authors, since the Greek prefix *iso* seems to imply perfect identity of genomes which may rarely or never be real. The Latin prefix *con*, on the other hand, denotes variable degrees of joint qualities without an implication of absolute identity. With reference to stocks produced by the occurrence of a single mutation within an inbred strain, on the other hand, where the new mutation is maintained by forced heterozygosis or by continued backcrossing, we consider the use of the term coisogenic justified and preferable to congenic.

The animals used in this analysis were derived from inbred strains, congenic stocks backcrossed to C57BL/6, and mutant stocks, all maintained at the Jackson Laboratory. Strain designations in the present report are in accordance with SNELL *et al.* (1960). Complete strain symbols are given in Tables 1 and 2; throughout the text, somewhat abbreviated forms are predominant. Mutant symbols, in accordance with GRÜNEBERG (1952), are as follows:

Linkage group I—chinchilla (c^{ch}), albino (c), and pink-eye (p)
II—dilution (d)
V—light-bellied agouti (A^w), black-and-tan (a^t), non-agouti (a), Danforth Short-tail (Sd)
VI—Caracul (Ca), Naked (N)
VII—Alopecia (Al), *Rex* (Re)
VIII—brown (b)
IX—Brachy (T)
XI—Microphthalmia-White (Mi^{wh})

TABLE 1

Inbred strains, congenic stocks and mutant stocks used in raising hybrid F_2 populations from outcrosses with 129/Re-dy or C57BL/6-dy

Strain or stock	Genotype	F_1 mated to
*129/Re-*dy*	$A^wA^wc^{ch}c^{ch}ppdydy$	.....
†C57BL/6-*dy*/Re	*aadydy*	.....
*BALB/c J	*bbcc*	litter mate
*C3H/He J	+	litter mate
*C57BL/6 J	*aa*	litter mate
*DBA/1 J	*aabbdd*	litter mate
*DBA/2 J	*aabbdd*	litter mate
†C57BL/6-Mi^{wh}/Re	$aaMi^{wh}+$	C57BL/6-*Dydy*
MI	$a^ta^tMi^{wh}+$	C57BL/6-*Dydy*
*AL/Ln	*aaAlAl*	C57BL/6-*Dydy*
CA	*aaCaCaSp*+	129/Re-*Dydy*
EI	*Re*+*Sd*+*Va*+	129/Re-*Dydy*
IC	*icic* (segregate *a*, *b*)	litter mate
JE	*aaffruie/ruje*	litter mate
YX	$a^ta^tMi^{wh}+N+T+$	C57BL/6-*Dydy*

* Inbred strains.
† Congenic stocks.

XII—ruby (*ru*), jerker (*je*)
XIII—Splotch (*Sp*)
XIV—flexed-tail (*f*)
XVI—Varitint-waddler (*Va*)
unlinked—ichthyosis (*ic*) and dystrophic (*dy*).

Host females bearing 129/Re-*dydy* ovaries (STEVENS *et al.* 1957), or, in the matings with stocks CA, JE and YX, C57BL/6-*dydy* ovaries, were mated to males of the strains and stocks listed in Table 1. Heterozygous F_1 animals were mated to litter mates in linkage tests of *dy* with recessive marker factors, and with either 129/Re-*Dydy* or C57BL/6-*Dydy* dystrophy carriers in tests with dominant markers. The matings of 129/Re-*dy* with BALB/c, C3H/He, C57BL/6, DBA/1 and DBA/2, respectively, were set up with the major goal of collecting 200 dystrophic hybrids from each group for a comparative longevity study. The primary object of the remaining combinations was to produce additional hybrid genetic backgrounds for the study of dystrophy expression and to provide data for the investigation of genetic linkage between *dy* and the various marker loci.

The reason for introducing *dy* into linkage test matings on two different genetic backgrounds, 129/Re or C57BL/6, consisted primarily in the need to prevent unfavorable pigment phenotypes from appearing in the segregating F_2. C57BL/6-*dy* animals were derived from a project of transferring *dy* onto a second highly inbred genetic background, in addition to 129/Re on which it had occurred. The transfer was carried out by continued back-crossing of host females bearing *dydy* ovaries to males of the C57BL/6 strain. The dystrophy carriers of the F_1 generation (C57BL/6-*Dydy*) were mated *inter se*, and black

TABLE 2

*Ratio of dystrophics (in percentage) segregating among F_2 hybrid populations from outcrosses of 129/Re-*dy *or C57BL/6-*dy *with several strains and mutant stocks*

Parent strain or stock used for *dy*-hybrid	Percent dystrophic	n†	Collection period‡
BALB/c J	22.3	1192	5/57– 5/59
C3H/He J	14.6	1796	1/57–11/59
*C57BL/6 J	23.2	1628	1/57– 8/58
*DBA/1 J	21.4	1158	5/57– 5/60
*DBA/2 J	20.4	1250	7/57– 9/58
*C57BL/6-*Mi^{wh}*/Re	23.2	198	1/59–12/59
*MI	26.4	326	10/58–12/59
*AL/Ln	20.6	257	1/59– 6/60
*CA	22.4	125	12/59– 7/60
*EI	22.9	414	1/60– 6/60
*IC	18.9	222	11/58– 9/59
*JE	24.0	438	11/59– 8/60
*YX	16.1	274	11/59– 6/60

* Populations used for linkage tests.
† Total number of F_2 classified.
‡ Month and year of first and last litter born.

dystrophic females of the F_2 generation served as ovary donors for the new backcross generation. The host females were obtained from mating C57BL/6-a^t with 129/J mice. In the resulting uniform F_1 hybrid, a^t and A^w served as dominant marker factors for possible offspring from regenerated host ovarian tissue.

Diagnosis of inbred as well as of hybrid dystrophic animals was based largely on the symptoms by MICHELSON *et al.* (1955). If animals of weaning age, i.e., about four weeks old, are held up by the tail, dystrophics are usually spotted at once by a characteristic clutching or crossing of the hind legs. The prevalent weakness of the hind legs is easily recognized by the lack of physical resistance when the legs are passively stretched out behind the animal, by the tendency of an animal to drag the stretched hind legs for a period rather than to walk on them immediately, and by the rapid exhaustion of dystrophics hanging from the rim of a glass jar by the hind legs. Occasionally a dystrophic animal displays a peculiar, apparently convulsive upward nodding of the head which jerks to an angle of 90° or over from the normal position. The upward jerking is accompanied by gasping. Animals exhibiting one or more of the symptoms described were diagnosed as dystrophic. Young litters were submitted to the same tests beginning at two weeks after birth, in order to determine the time and sequence of onset of clinical symptoms.

Segregating F_2 litters were classified four to five weeks after birth. In a few cases, positive diagnosis for muscular dystrophy had to be postponed for one or two additional weeks. Young animals that died before weaning date were included in the segregation records if classification for each of the loci being tested for linkage was accomplished before death. All dystrophic animals (*dydy*) in the hybrid F_2 populations from matings of 129/Re-*dy* with BALB/c, C3H/He, C57BL/6, DBA/1 and DBA/2 were saved for determination of lifespan.

RESULTS

For the convenience of discussion, the results are arranged into three groups and presented as (1) dystrophy ratio, (2) clinical expression and (3) linkage studies. The lifespan data, which are incomplete at the time of reporting, will be discussed in full in a separate communication.

Dystrophy ratio: Hybrid F_2 populations segregating for the *dy* factor have been produced from 13 different types of parent combinations (Table 1). Dystrophic phenotypes have appeared in all these populations. Moreover, they have in all cases segregated in ratios approximating the theoretical expectation of 25 percent for inheritance by a single recessive factor. The data are summarized in Table 2. The small but persistent deficiency of dystrophics is presumably due either to selective preweaning mortality of dystrophic individuals or to incomplete penetrance of *dydy*.

The most critical evidence to demonstrate that the dystrophy factor is an hereditary entity was obtained from the continued backcrosses of *dy* into the inbred C57BL/6 genetic background. Through the six backcrossing cycles that are completed at the time of reporting, only the third backcross, with 17.6 percent, has

a dystrophy ratio significantly different from the expected 25 percent (Table 3). This low ratio appears erratic, however, and may be attributed to selective mortality of dystrophics or to some other nongenetic interference. The brief period during which offspring from each backcross generation were accumulated, e.g. November 1958 to April 1959 for the group in question, suggests the possibility of erratic results due to seasonal exogenous factors. The persistence of nearly 25 percent incidence, in spite of a change of approximately 98 percent of the original 129/Re genotype to that of C57BL/6, strongly supports the interpretation that muscular dystrophy in the mouse is transmitted as a single factor.

It is probable, however, that the aberrant dystrophy ratio of only 14.6 percent in the large F_2 hybrid group from a 129/Re-*dy* × C3H/He outcross (Table 2) is caused by genetic modifiers. The dystrophy incidence in the data from this outcross is even lower than from the exceptional third backcross generation of *dy* × C57BL/6, the sample is much larger, and the degree of significance very high. Moreover, the C3H F_2 hybrids were collected continuously between January 1957 and November 1959, so that seasonal factors, which would tend to be compensated, would not be expected to lead to such a highly erratic result. If excessive preweaning mortality of dystrophics were responsible for their observed deficiency in this F_2 population, the total preweaning mortality would be raised. However, total preweaning loss in the C3H F_2 (25.4 percent) is not significantly greater than in three other F_2 outcross populations (129/Re × BALB/c 24.4 percent, 129/Re × DBA/1 23.5 percent and 129/Re × DBA/2 23.9 percent) which show higher incidence of dystrophy (Table 2). It is much more probable, though not absolutely proven, that the deficiency of dystrophics on the 129/Re × C3H F_2 population is due to reduced penetrance of the *dydy* phenotype on this particular genetic background.

Additional positive evidence favoring the view of background factors influencing dystrophy penetrance is derived from experiments still in progress with a *dy* × MI hybrid group (LOOSLI and SOUTHARD, unpublished). Dystrophy symptoms in animals from this cross are almost always distinct at two weeks of age, and their further progress is unusually rapid. In several statistically homogeneous

TABLE 3

Ratio of dystrophics (in percentage) segregating from consecutive backcrosses of dy *to inbred C57BL/6*

Backcross generation	Percent dystrophics	n*	Collection period†
1	23.2	1628	1/57– 8/58
2	23.4	111	6/58–10/58
3	17.6	170	10/58– 4/59
4	24.1	257	3/59– 8/59
5	25.6	242	7/59– 1/60
6	27.5	40	2/60– 4/60

* Total number of segregating animals classified.
† Month and year of first and last litter born.

groups of hybrids F_2 and F_3 populations, dystrophy segregation is significantly higher than 25 percent and reaches 32 percent ($P = 0.01$) in one group of 266 individuals. It is our working hypothesis that this abnormal segregation, which cannot be explained by postnatal selection, is due to genetic modifiers. The genetic action of this system of modifiers is not yet understood, and there is no indication revealing its physiological mode of action.

Clinical expression: In its major characteristics, the clinical manifestation of *dydy* is very similar on the various backgrounds under investigation. A pronounced weakness of the hind legs is the prevalent symptom in the 129/Re-*dy* and C57BL/6-*dy* as well as in all the hybrid dystrophics. The first indication of weakness frequently consists of a slightly irregular, waddling gait which may be visible as early as twelve days after birth. The positive identification of dystrophics is based principally on the dragging of the hind legs. It may be induced by forcing the legs into a stretched position as early as two weeks after birth. At four weeks of age, spontaneous dragging may be observed occasionally, and in most of the cases it can be induced by manipulation. (Diagnosis of a very few animals, which later show the dystrophy symptoms, is still questionable at four weeks). At older ages, the weakness of the hind legs progresses further until they are practically immobile and useless. In this final stage, which may be reached at about four to five months, the hind legs are extended backward and bent upward. Meanwhile, the animal retains the forelegs in a usable state up to its maximum age of over two years.

The development of clinical symptoms was studied by routinely checking young litters from the five largest hybrid F_2 populations (Table 2) at weekly intervals up to weaning age. Variations between hybrid groups were thereby noted by subjective observation. The progressive sequence of dystrophy symptoms follows the same general pattern in all five groups. Except for the C3H hybrids, the mean age at which dystrophy could be positively diagnosed in a sample of 50 dystrophic animals lay between 19 and 20½ days after birth, with a range from 12 to 35 days. In a sample of 70 dystrophic C3H hybrids, however, the mean age of diagnosis was 28½ days, with a range from 15 to 93 days. On 19 animals from this group, final diagnosis could not be made until more than 30 days after birth, and in four among these 19 animals there was no indication of dystrophy up to 30 days.

By subjective standards, the clinical expression of dystrophy in C3H hybrids was less severe than in other hybrids. A spontaneous display of typical symptoms was rare up to weaning age. Usually the animals had to be overexercised before the muscular weakness was noticeable. Among the remaining four groups, clinical expression appeared slightly more pronounced in the C57BL/6 hybrids than in the other three. At weaning age, they were usually diagnosed without manipulation.

A qualitative difference of a dystrophy symptom between hybrid *dydy* and 129/Re-*dydy* was found in the pattern of convulsive head-jerking. Where in the 129/Re-*dy* the pattern is always the head-jerking and gasping described above, in hybrids it is frequently modified to jittery head motions, with the front

paws "rubbing the nose" as if to hold the head from shaking. The typical jerking has also been observed occasionally in hybrids, however, so that it cannot be considered highly specific for 129/Re-*dy*.

Before weaning, 129/Re-*dydy* animals frequently suffer from an eye infection which appears to be conjunctivitis. The infection responds poorly to oral terramycin treatment, but local application of bacitracin appears to be beneficial. Without treatment the infection usually becomes chronic or may lead to an abscess destroying the eye. This infection is rarely found in hybrids, probably because they are more resistant due to "hybrid vigor."

Linkage tests: The segregation in F_2 of the mutant loci listed in Table 1 was used to test for possible genetic linkage with the *dy* locus. The linkage observations involved a total of 16 marker loci representing 11 linkage groups (GREEN and DICKIE 1959), and the independent locus *ic*. A^w, *p* and c^{ch} were tested with *dy* in coupling, all other markers with *dy* in repulsion. Only Splotch *(Sp)* could not be used for testing because its heterozygous expression was lost in the F_2.

No convincing evidence of linkage resulted from this series of linkage tests. The possibility of loose linkage to the *p* locus (pink-eye, linkage group I) or to the *Sd* locus (Danforth's short tail, linkage group V) could not be ruled out on the basis of present data, however, and needs further testing. The remaining 15 loci have given no evidence of linkage with *dy*.

DISCUSSION

Dystrophic mice have been obtained in frequencies approaching theoretical expectation among the hybrid F_2 generations of all the 13 strains and stocks of mice into which the original 129/Re-*dy* has been crossed. Moreover, upon continued backcrossing of *dy* onto the inbred C57BL/6 genetic background, the ratio of dystrophics has satisfied theoretical expectation despite the very high degree of "dilution" of the original 129/Re background. The conclusion that hereditary muscular dystrophy in the mouse is caused by a factor behaving as a single, recessive mutation is thereby corroborated.

In all the hybrids so far observed, the clinical expressions of the dystrophy are essentially uniform. The pronounced weakness of the hind legs, contrasted to a remarkable fitness of the front legs, was observed without any exception. The variability of the syndrome was confined to quantitative characteristics, such as time of clinical onset, and the ranges of variability in different groups overlapped each other widely. The dystrophy syndromes in different hybrids clearly present one clinical entity, corresponding to one underlying hereditary entity.

This situation parallels the cases of human hereditary dystrophies where three fairly distinct clinical entities, described as Duchenne type (sex-linked recessive), limb-girdle type (autosomal recessive) and facio-scapulo-humeral type (autosomal dominant), appear to be caused by different factors (BECKER 1957, 1958; CHUNG and MORTON 1959). That there are at least three hereditary factors is indicated by three different modes of inheritance. The possibility that

various distinct mutations may cause only one clinical pattern still exists. But it is clear that each dystrophy mutation affects muscles selectively in a characteristic pattern of regional specificity, which persists through consecutive generations of each pedigree and is not affected by altered genetic backgrounds. With the large number of hybrid mouse populations now investigated, the same constancy of regional specificity can be stated for the action spectrum of the *dy* factor in the mouse.

Some discrepancy between mouse muscular dystrophy and each of the three major human dystrophy types is consistent throughout the hybrid populations. In all groups of mice so far observed, dystrophy resembles the human Duchenne type in its clinical aspects, including differential involvement of the hind legs. However, unlike the Duchenne type, it is transmitted as an autosomal recessive. Although mouse muscular dystrophy and limb-girdle type dystrophy in man are both caused by autosomal recessive factors, the clinical expressions of the two disorders are markedly different. In the mouse, severe posterior limb impairment always precedes the mild anterior limb weakness. In limb-girdle dystrophy, on the other hand, the upper limbs may become involved first, and in advanced stages they are severely affected. The question whether the disorders in the mouse and in humans are caused by analogous factors affecting the same target area in a similar way, or if the mode of transmission of homologous loci has become different in the rodent and in man, cannot be resolved at present. In utilizing the dystrophic mouse as a model in clinical research, it should therefore be remembered that it is still unknown how closely the model actually fits any of the human disorders.

In the cases of abnormal dystrophy ratios presented above, it was suggested that genetic modifiers were responsible for significant deviations from expectation. Among the various mechanisms that are known to cause abnormal segregation, and that may be attributed to controlling hereditary factors, a modified degree of penetrance of *Dy* versus *dy* appears especially feasible. In this hypothesis, high incidence would be caused by the occurrence of phenotypically dystrophic, heterozygous *Dydy* animals, while incomplete penetrance of *dy* even in homozygous doses would lead to low incidence. We observed lessened expression of the syndrome among dystrophics of the low-incidence *dy* × C3H hybrids, and on the other hand very pronounced expression in the high-incidence *dy* × MI hybrid group. This observation lends support to the concept of modified penetrance, even though penetrance and expressivity are not invariably correlated.

It is interesting in this connection to remember that investigations of sporadic cases of human limb-girdle dystrophy have led to the same assumption of occasional expression of dystrophy in the heterozygous state (MORTON and CHUNG 1959). CHUNG, MORTON and PETERS (1960) reported on the case of a carrier mother of two Duchenne boys who developed typical limb-girdle dystrophy symptoms. They interpret this as a case of dystrophy expression in the heterozygous state. Among the mice of various uniform F_1 hybrid genetic background types which from their ancestry were known *Dydy* heterozygotes, however, no animal has yet developed the characteristic dystrophy syndrome. The expres-

sion of dystrophy in heterozygous carriers, a phenomenon presumably discovered in humans, therefore still needs to be demonstrated by direct evidence in the mouse.

SUMMARY

Thirteen different populations of heterogeneous hybrid dystrophic mice were produced by crossing the *dy* mutation from the inbred 129/Re strain, where it had originally occurred, into other stocks and strains of mice. All results clearly supported previous reports that mouse muscular dystrophy is transmitted by a single autosomal recessive factor. Despite the heterogeneity of the dystrophics obtained, the clinical symptoms of the disease were not markedly different from their expression in inbred animals. It is concluded that the characteristic pattern of mouse dystrophy, in analogy to each of several distinct clinical entities known in man, is subject to effects of genetic modifiers only to a minor degree. Results from a continued backcross of *dy* to the C57BL/6 background further confirm this conclusion. Evidence was found, however, suggesting genetic modification of the penetrance of mouse muscular dystrophy. No definitive evidence of genetic linkage of *dy* to any of the mutations used in the experiment was found. Comparative aspects of hereditary muscular dystrophies in man and in the mouse are discussed.

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